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Darwin's death in South Kensington

What is the British Museum (Natural History) for? Research? Public enlightenment, even entertainment? Or should it be, as Dylan Thomas said in another connection, a "museum that should be in a museum"? In spite of the stream of correspondence in the past several months, itself a measure of the museum's claim on public esteem and even affection, we are none the wiser. The museum, now at the beginning of its centenary year, has not replied to the several issues which have been raised since Dr Colin Patterson's cool comment on the role of cladistics in taxonomy (*Nature* 288, 430; 1980). Halstead (this issue, page 742) is right to draw attention to this deafening silence.

The issues are not, after all, so complicated. Cladistics is a technique used in systematics for the classification of organisms that may or may not belong to distinct species. Cladism, of which Halstead is a well known enemy, is presumably the belief that cladistic analysis yields meaningful results. Halstead's original complaint against the museum was that a new public exhibition at the museum on "fossil man" is shot through with cladism to such a degree that familiar relationships between fossil human forms are obscured, which only went to show that the museum had been captured by Marxist ideology. The second charge, always a red herring, cannot be sustained. The public face of the museum is trendy, not revolutionary. The questions which have been raised, and which the museum must in the public interest answer, are not nearly as grand but are, by the same test, not so easily ignored.

Even the row there has been about cladism in public exhibitions is probably only a symbol for a more mundane but more important discontent — the museum's exhibition policy that has been evolving for the past five years or so. Waterhouse's Victorian pile has been a hive of activity throughout that period. The dinosaurs and stuffed birds have taken a back seat, or have been hidden away in back rooms, to make way for much more jazzy exhibitions. Although some of these are second-rate (that on ecology), simple inspection shows that others (that on human biology, subtitled "An exhibition of ourselves") fascinate and delight the crowds of visitors, many of them young, who are forever pressing buttons to provoke yet another physiological model into action. Nobody disputes the public demand for this kind of exhibition and there is even a possibility that it may assist in the education of the young and the general public. The practical issue is whether the museum should be encouraged, or even allowed, to fill itself with "exhibits" designed to "present the whole of modern biology" when the acknowledged consequence will be the replacement of "almost all" of the existing exhibitions.

This quarrel has been simmering for a long time, but the museum's dilemma is not negligible. Its postwar managers inherited a labyrinth of poorly lit galleries in which specimens of various kinds were so crammed together that their identity could hardly be discovered, especially if the ink had faded on the labels. The choice was to make the existing exhibition intelligible or to embark on a more ambitious policy. In 1972, when the pressures on public funds were magically almost imperceptible, the die was cast and the decision taken to remodel the whole museum. Since then, the museum has been constantly in hot water over its building plans. (The latest scheme for rebuilding the back of the museum, in the process changing the outline and the roof profile, confirms the suspicion that only public authorities can now commit vandalism openly.) Two questions persist. It there is a public need in London of a popular museum of science, why should its creation mean the end of the more scholarly exhibition created by the Victorians? The truth is that there is a need for

both. And if the museum is irretrievably launched on its new policy of public education, how sensible is the course it is following?

The museum's showing so far is not encouraging. The latest brochure from the museum starts from the view that learning should be enjoyable. There is nothing wrong with that, even though the repetition of the jingle "learn and enjoy" may make many serious teachers wince. The danger, which many popular museums of science have discovered, is that enjoyment often interferes with learning. Pressing buttons to make things happen is an absorbing occupation at most ages, but is not by itself an educative process. In reality, the new exhibitions at the Natural History Museum, novel though they are for central London, differ only in scale from others to be found elsewhere in the world. It would be easier to believe that the museum is embarked on a worthwhile course if there were signs that it had seriously set out to break new ground in the public exhibition of science. Is it too late to look for signs of redemption in this respect? The new exhibitions promised for the summer may provide some clues.

The charge that the museum's new exhibitions are shot through with heresy, overstated though it may have been, also needs to be answered more categorically than in the past few weeks. Again there is right and wrong on each side. In the latest brochure, the popular euphemism for cladism is the phrase "the groups-within-groups classification", presumably a synonym for species. There is also the following passage: *Biologists try to reconstruct the course of evolution from the characteristics of living animals and plants and from fossils, which give a time scale to the story. If the theory of evolution is true . . .* If the words are to be taken seriously, the rot at the museum has gone further than Halstead ever thought. Can it be that the managers of the museum which is the nearest thing to a citadel of Darwinism have lost their nerve, not to mention their good sense? Or is it that somebody has calculated that the museum will increase its annual intake of visitors by enticing in scoffing creationists?

This passage nicely illustrates what has gone wrong at the museum. The new exhibition policy, the museum's chief interaction with the outside world, is being developed in some degree of isolation from the museum's staff of distinguished biologists, most of whom would rather lose their right hands than begin a sentence with the phrase "If the theory of evolution is true . . ." Nobody disputes that, in the public presentation of science, it is proper whenever appropriate to say that disputed matters are in doubt. But is the theory of evolution still an open question among serious biologists? And, if not, what purpose except general confusion can be served by these weasel words?

The most urgent need at the museum is to restore the once close relationship between the museum's scholarship and its external face. It is demeaning that many of those working as scientists at this distinguished place should not have been persuaded to harness their scholarship to the brave new policy. No doubt, if they had been more closely involved, the pace of development would have been slower and the museum might have been dissuaded from its promise to put "the whole of modern biology" on display. That, if the managers think about it, would be an advantage. For all its distinction, the museum is not equipped for such a task. What it can do well, as in the past century, is to tell the absorbing tale of natural history. It should bend its mind to that, not to worrying about cladism. It should beware of selling out on Darwinism. And it should say, from time to time, what it is about. Hitherto, the museum has been too secretive.



New Pentagon rules on overseas students

Secrecy threat to sensitive research plans

Washington

Restrictions on the use of foreign research students on non-classified research projects sponsored by the Department of Defense are in the offing. This prospect stems from the requirement, recently emphasized by Congress, that the multi-million dollar research programme sponsored by the department in very high speed integrated circuits should be strictly controlled to prevent the export of technical data of potential military value.

Pentagon officials say they are "bending over backwards" to minimize interference with the freedom of university research in applying controls. These controls were introduced in 1972 as the International Traffic in Arms Regulations (ITAR) to cover the export of unclassified technical data. Many academics, however, see a danger of renewed threats to academic freedom previously raised by secret military research on university campuses.

Because of the uncertainties which abound, the Defense Department has issued guidelines to both universities and private contractors carrying out sponsored research on integrated circuits. Where research can be classified as "process development" and is likely to lead to a specific improvement of military technology, controls will apply and it will be necessary to obtain an export licence from the State Department before the research is even discussed with a foreign citizen. But where the research can be classified as the "general pursuit of knowledge", with no particular application in mind, then controls will not apply.

Pentagon officials admit that there is a "grey area" dividing the two, particularly in materials science research and other areas closely linked to improving the efficiency of semiconductor systems. The Defense Department has told universities of its "preference" that foreign students should not be employed on integrated circuit programmes, but that the programme office will "make a decision based on the nature of the research" where restraint is not feasible.

The consequences could be significant. The proportion of foreign research students in US universities has risen dramatically in the past few years to about 50 per cent — and is particularly high in fields such as computer science, where first degree graduates can command large salaries in the private sector.

Several universities are concerned that strict interpretation of the regulations could reduce flexibility in research

programmes, especially since the Department of Defense is the major supporter of this line of research. There is also mounting anxiety that the regulations might be abused. They are written so broadly that it is a federal crime to discuss with a foreign scientist any research result which might improve the "state of the art" of US military technology without prior approval by the State Department.

These fears have been reinforced by the National Security Agency's attempts to restrict the dissemination of cryptography research results obtained by Dr George Davida of the Georgia Institute of Technology (see *Nature* 19 February, p.621). Integrated circuit research is less sensitive, but government officials could theoretically use the regulations to close down semiconductor research in universities, on the grounds that, almost by definition, such research must advance the state of the art.

Commercial contractors for the Defense

Department are also worried, since a strict interpretation of the regulations could prevent them from communicating research data, even if unclassified, to overseas affiliates.

The Defense Department, well aware of such objections and keen to avoid fresh campus confrontations such as those during the anti-war demonstrations of the 1960s, hopes that its new guidelines will convince universities of its concern for the freedom of research. Its proposals have been circulated through the Association of American Universities, a group of top US research universities, and will be discussed at a meeting of university and government officials in Washington next week.

Faced with other financial problems, universities are keen not to rock the boat by rejecting the new requirements as unreasonable. But many are wary of what they are letting themselves in for — hoping that it is not the thin end of an increasingly sticky wedge.

David Dickson

British gene company opens books

Britain's national biotechnology company, Celltech, has begun marketing its first product: the monoclonal antibody to leukocyte interferon which, fixed to beads in a fractionation column, can purify interferon 5,000-fold in a single step. But since the discovery (see *Nature* 285, 446-458; 1980) fell before November 1980, when Celltech negotiated rights to Medical Research Council biotechnical inventions, the National Research Development Corporation will retain a slice of the action. Profits on sales will be divided between Celltech, the corporation and the council.

The researchers who created the cell line, Dr David Secher of the MRC Laboratory of Molecular Biology, Cambridge, and Professor Derek Burke of the University of Warwick, also stand to gain but by different routes. In Burke's case, the standard revenue-sharing agreement will give the University of Warwick half the development corporation's profit; and Burke and colleagues in his laboratory will receive half of that, less the university's costs in running the laboratory for the relevant period. Secher, on the other hand, must look to his employer, the Medical Research Council. The council's agreement with Celltech allows for some reward for the researcher, but it is not clear at this stage how much. There is a standard arrangement for "inventors" in which a joint council-corporation panel meets every three years (the next meeting is in December 1982) to distribute largesse to those who have contributed to the council's coffers by work "outside normal duties". (In 1979, £130,000 was distributed to 30 inventors in amounts ranging from £40 to some thousands of pounds.) The catch is that many commercially valuable

inventions — like monoclonal anti-interferon — fall within normal duties, and so in principle outside the inventors' scheme. Nevertheless, the council says it will interpret the phrase liberally in future.

In the end, the central issue is not how the loot will be divided but whether there will be any loot at all. The council hopes that the fledgling and hungry Celltech will be more assiduous than its traditional partner, the National Research Development Corporation, in exploiting inventions. Proposals are already in hand with a number of potential customers interested in the purification and radioimmunoassay of interferon using the antibody. However, Hoffmann la Roche is also believed to have developed similar cell lines, so there may be commercial competition.

Then the question will be how good is the Celltech line? There is more than one kind of interferon, and Celltech's antibody is strictly an antibody to a single interferon, one from the group of interferons supplied by the Wellcome Laboratories (consisting of 8 to 16 proteins) which formed Burke and Secher's starting material. Nevertheless, says Burke, evidence is accumulating that their antibody will bind to similar sites in a number of related interferons, so its specificity may not be unduly narrow. The initial quantities of antibody will be produced in Cambridge. Ultimately production will move to Celltech's new laboratories in Slough. Celltech expects a modest market for interferon for clinical trials over the next few years, with the promise of a much larger market if interferon proves to have therapeutic value.

Robert Walgate

Celltech's deal with MRC

The Medical Research Council revealed last week the broad outlines of its five-year agreement with Celltech, signed on 6 November last. The agreement raises perturbing prospects of delays in the disclosure of scientific information. It concerns work done by researchers directly employed by the council and not that of its grant-holders employed by universities.

The council has undertaken to inform Celltech of commercially interesting discoveries by its researchers in fields related to biotechnology before the work is published. (In other fields, the traditional relationship with the National Research Development Corporation will be maintained.) The council says that the freedom of its research workers to communicate with other academic scientists will not normally be impaired, but occasionally publication would have to be delayed "for a short period" while a decision is taken on patenting.

Dr Sydney Brenner, director of the Laboratory of Molecular Biology and a member of Celltech's Science Council, is unconcerned. Delays will not be serious, and the issues involved are less difficult than those which arise when individuals have links with commercial companies.

In return for the right to information (and ultimate exploitation), Celltech will give the council a "substantial proportion" of any royalties or profits that ensue. The council will place this money in a separate "Celltech fund". When the fund reaches a sensible level, a sub-committee will call for proposals from both universities and council establishments on how it should be spent. In the early stages, when the fund is limited, grants will be restricted to fundamental research in areas related to biotechnology. If the money rolls in, the scope of the fund will be widened.

If, however, the sum in the fund rises beyond a fixed limit, which has been agreed between the Department of Education and Science and the council, but which neither will reveal because of its quasi-commercial nature, the dispensation that allows the council to accept money from Celltech will have to be renegotiated. Hitherto, the arrangement between the research councils and the development corporation has been that the research councils can receive no such payment.

Robert Walgate

Polish universities

Sit-in complete

Last week, Poland's month-old student sit-in in Lodz ended with the signing of a wide-ranging agreement on academic autonomy. The students' action had begun over alleged delays by the Ministry of

Science, Higher Education and Technology in registering the new Independent Students' Association (NZS). Later, as the Lodz University students were joined by those from the Lodz Technical University and Medical School, and then by students in Poznan, Krakow and Warsaw, their package of "postulates" grew to more than fifty issues.

The problem of getting NZS registered had been dragging on since last November, when a limited sit-in at Warsaw University won a promise that the ministry would produce the necessary regulations by 20 December. Difficulties with the statutes of NZS were finally resolved last week, by the inclusion of a clause stating that NZS would abide by the constitution of the Polish People's Republic, but without specific mention of the "leading role" of the Party.

The major part of the Lodz Accord, however, sums up progress on the promised liberalization of Polish academic life. Several clauses therefore simply reiterate what has already been conceded. These include: the abolition of "reserved" places at universities, granted at the discretion of rectors or ministry officials to candidates whose performance in the entrance examinations did not merit their admission; university courses to be decided by the staff of the universities concerned; staff appointed on academic merits only; student participation in university government, and the election of university rectors by the academic senate in secret ballot.

Of the new demands raised at Lodz, the most radical have been dropped, including the reduction of military service and the abolition of compulsory courses on Marxism. (Some choice in "sociopolitical subjects" has, however, been conceded.) One major gain for the students concerned language courses. This had been strongly opposed by the minister, Dr Janusz Gorski, who had argued that a university is not geared to teaching foreign languages *ab initio*. Nevertheless, there will now be a wider choice of languages to be studied as subsidiary subject, in effect ending the compulsory Russian which many science students in particular resented. Having had several years of Russian at school, they say they would like to study a Western language, to have greater access to current research literature.

Access to information should also be facilitated by clauses promising larger editions of textbooks (most students now have to make do with secondhand texts), more foreign currency for buying foreign journals and research apparatus and more open availability of existing literature.

A new system of student grants "more in tune with the principles of social justice" is promised for 15 May, when it will be submitted to the students for consultation. By May, too, the detested "practical training" in manual labour will be replaced by paid voluntary physical work.

Student participation in university senates and faculty councils is set at 20-33 per cent, the student delegates to have full voting rights except in the awarding of academic degrees and titles.

The students also seem to have won their demand that the police should not be able to enter university premises at will. This clause, however, will presumably have to undergo some modification before the new legislation on higher education finally comes before the Sejm (Parliament), if campuses are not to provide an unintended sanctuary for, say, a purse-snatcher on the run. Inclusion of the clause, however, seems to reflect just how far the authorities are prepared to accept the concept of university autonomy.

What is clearly less acceptable, however, is the prospect of further unrest. Before finally approving the NZS statutes, therefore, Dr Gorski made sure that they contained some limitation on future militancy. The ministry's revisions laid down that strike procedures would follow the pattern of the "Solidarity" statutes, with the additional proviso that a sit-in can be proclaimed only by the majority decision of a specially convened meeting of a students' organization, in consultation with the authorities of the higher educational establishment(s) concerned.

Vera Rich

US science budget

Reagan's way

Washington

In the absence of any explicit science policy — and, indeed, of any official presidential science adviser — the new Reagan Administration has adopted a "back-to-basics" approach in deciding where the budget axe should fall on federally sponsored research programmes.

Last week, Mr Reagan submitted to Congress the cuts which he is proposing, as part of a "national program for economic recovery", in the budget recommendations made by Mr Carter last month.

As expected, the main cuts will fall on those additional areas of responsibility which the federal government has taken on in recent years but which Mr Reagan is suggesting should be returned to individuals, to states or to private corporations and other institutions.

In the universities, for example, he proposes to eliminate a new fund for modernizing research equipment which Mr Carter had proposed creating in the National Science Foundation, with an initial budget of \$75 million. Mr Reagan also wants to cut federal support for biomedical research institutions previously provided through the National Research Service Awards.

The Department of Commerce's National Oceanic and Atmospheric Administration (NOAA) would see its budget cut by more than two-thirds,

Threat to solar mission

Washington

European scientists could suffer directly from Mr Reagan's proposed budget cuts through a recommendation that the United States reduce its commitment to the international solar polar mission, a two-spacecraft programme run jointly by the National Aeronautics and Space Administration (NASA) and the European Space Agency (ESA).

Although no details of the reduced commitment have been officially announced, the White House is said to have suggested that NASA should stop work on its own spacecraft. This would effectively eliminate half of the project, and seriously reduce its scientific value, much of which depends on the simultaneous collection of data from the two spacecraft as they follow polar orbits around the Sun.

NASA and ESA officials are now discussing the implications of Mr Reagan's proposal, which could still be modified before the details of the new budget are announced on 10 March. The solar polar mission was threatened with termination by Congress last summer, but survived after vigorous intervention by the State Department and the Office of Science and Technology Policy.

Under the budget proposal submitted to Congress by President Carter last month, the two spacecraft would be launched from the space shuttle, using a modified Centaur launcher as a substitute for the delayed inertial upper stage, early in 1986.

David Dickson

including an end to grants to states to protect their coastal zones, a 50 per cent reduction in support of college research under the Sea Grants programme, and the deferment of the National Ocean Satellite System (NOSS).

As for the private sector, a firm belief that the federal government should not interfere with the mechanics of the marketplace is reflected in substantial cuts to the Department of Energy's research and development budget. These would eliminate many of the department's efforts to demonstrate the commercial potential of new energy technologies, such as synthetic fuels, coal liquefaction and solar energy; but the aim is to maintain a basic commitment to long-range research projects considered too expensive or too risky by the private sector.

Public reaction to the proposed cuts from the scientific community has so far been muted. This is partly because precise details of where the cuts will fall will not be announced until 10 March and partly because there is little at present to be gained in Washington by speaking out against massive cuts in federal expenditure. Privately, however, laboratory chiefs and university presidents are already pulling all

the strings they can, both within Washington's scientific establishment and among their congressional allies, to protect their own research programmes.

Some may already have been effective. A proposal from Mr David Stockman, director of the Office of Management and Budget, to eliminate the National Aeronautics and Space Administration's Galileo mission to Jupiter was removed from the President's message to Congress. Given a decision to defer the Venus Orbiting Imaging Radar, this would have virtually wiped out all future planetary research at NASA's Jet Propulsion Laboratory in California, Mr Reagan's home state.

On other proposals, there are bitter fights in prospect. Most of the projects that Mr Reagan is proposing to defer have been argued for by the scientific community strong and hard. These include NASA's gamma-ray observatory (already approved for funding by Congress), the National Science Foundation's 25-metre millimetre-wave radioastronomy dish and the NOSS satellite system.

Funding for space transportation systems will be maintained at a level adequate to cover the costs of the space shuttle, at the expense of slower development for Spacelab, and the rescheduling of space science flights — Galileo, for example, is likely to be shifted back from a 1985 to a 1986 launch. There will also be no funds for the solar electric propulsion system for which, in the absence of a Halley's comet mission, no applications have been approved.

At the National Science Foundation, budget restrictions will, as previously rumoured, be concentrated on programmes that are "narrowly focused or of less immediate priority" — such as innovation in small businesses and international scientific efforts — as well as on new initiatives in science and engineering education, and on research in the behavioural, social and economic sciences.

In contrast, there would be no reduction in the previously proposed 17 per cent increases for research in the mathematical and physical sciences, or the 20 per cent increase for engineering research. Both are considered by the new Administration to be "of relatively high importance to future technological advancement and to the long-term health of the nation".

In energy research, Mr Reagan is proposing a reduction of \$40 million in the \$607 million which had been suggested for basic energy sciences. Details of how this cut will be distributed are still being discussed. Most of it is likely to fall on high-energy physics, which accounts for two-thirds of the total, and will suffer the delayed construction of new facilities.

Biomedical research has been left relatively untouched; where the previous Administration had suggested a relatively modest 9 per cent increase for the National Institutes of Health (NIH), Mr Reagan is

suggesting a slight reduction in both 1981 and 1982 funding that will reduce this to 6 per cent. Much of the saving would come from reduced payment to educational institutions for NIH research training, which the Administration says would eliminate the practice of paying more to an institution for a federally supported trainee than would be charged for those who are not federally supported.

The Administration says that even though its proposed new budget for NIH would not fully cover the projected inflation rate — and that real reductions below the present base will therefore have to be made across all NIH institutes — it is committed to maintaining a substantial number of new research awards. It is therefore likely to continue the previous Administration's strategy of focusing on competitive project grants, rather than programme grants or intramural research.

David Dickson

UK nuclear energy

CEGB sheepish

The British nuclear industry is outwardly unruffled by criticisms from the House of Commons Select Committee on Energy last week (*Nature* 19 February, p.621). The general response is that the committee has misunderstood many of the issues. The strongest reaction is to the committee's criticism of the government's 1979 statement on nuclear power, which the committee took to be a commitment to build one nuclear station a year for the coming decade. This, it is said, was never the intention, so that the committee's recommendation that each reactor should be judged on its merits is already part of public policy.

The Central Electricity Generating Board (CEGB), the organization most sharply criticized, was the most sheepish last week. There is plainly some foundation for the charge that it had not been forthcoming with up-to-date estimates of cost and electricity demand. On the complaint that nuclear power stations are 34 per cent more expensive to build in Britain than elsewhere, Mr Glynn England, chairman of the board, is to meet nuclear suppliers and subcontractors to find ways of cutting costs and improving productivity on nuclear plant sites.

Mr England does not, however, accept the committee's view that the ordering of a second pressurized water station should be delayed for six or more years until the first, now being designed for the Sizewell site, is operating. Detailed studies and a public inquiry should provide enough information for the board to decide whether subsequent reactors should be based on pressurized water (as at Sizewell) or gas-cooled technology.

The Nuclear Installations Inspectorate also says that the committee has misunderstood its role. In particular, it

says there would not be enough work for a full-time ultrasonics expert, recommended by the committee for testing pressure vessels, and that this and other work will be contracted out when it lacks the appropriate experts on its own staff.

The inspectorate is nevertheless still short of nuclear inspectors. Thirteen posts out of a total of 102 remain to be filled and salaries are a problem. Although nuclear inspectors' pay is as good as or better than that of other inspectors, it is below that in the industries from which it has to recruit. The problem — that the inspectors' salaries are linked to civil service pay — cannot be solved by removing the inspectorate from the Health and Safety Executive, which the inspectorate says would complicate licensing procedures.

The committee is said also to have misunderstood the role of the chief scientist at the Department of Energy and of the UK Atomic Energy Authority in advising the government on nuclear matters. The Department of Energy says that its chief scientist is responsible for nuclear advice but that there were exceptions when Dr Walter Marshall held

the post as well as that of deputy chairman of the UK Atomic Energy Authority. Dr Marshall's version of this difficulty is different. He said last week that during his spell as chief scientist at the department, he felt no conflict of interest but, with the minister's agreement, meticulously kept the chairman of the authority informed of the advice he gave on nuclear matters.

The response so far to the report has been laconic and avoids detail. A more considered reply is likely to be published by the Department of Energy some months from now. Another contribution is likely to come from the Monopolies and Mergers Commission when it makes its views known on the structure of the electricity supply industry on 2 March.

Judy Redfearn

UK science research On the move

The Science Research Council's attempt to foster mobility among British academics has made a modest beginning. The first four awards under the council's Special

Replacement Scheme were announced last week. The scheme is designed to release senior academics from routine duties for five years, replacing them with younger people, usually postdoctoral researchers. The four awards will be followed by a further eleven before July, after which the council hopes to make ten awards a year.

The first four awards go to people who will be released from some or all of their administrative and teaching commitments for up to five years, enabling them to concentrate on their research interests. Each of the four departments is advertising a vacancy for a lecturer, whose appointment will be financed wholly by the Science Research Council for the first five years of his tenure. In every other respect, however, those appointed will be fully-fledged members of the academic staff.

Here the similarity ends. Some of the senior academics will return to their original position after five years. During that time, the department, which has guaranteed tenure to the new appointee, will have either found money elsewhere or lost a member of staff (by foul means or, more probably, fair). In other cases, the senior person will himself be retiring.

The Science Research Council, which has designed the scheme for flexibility, makes no stipulation about the areas of research involved, although it does intend to be represented on the selection boards for new appointees. In the cases so far announced, one professor should gain a member of staff in his own field of research, while another's department is to advertise for applicants for any of its research areas. Much discussion takes place behind the scenes between the council and the university concerned, and Sir Harry Pitt (ex-vice-chancellor of the University of Reading) is go-between and honest broker for the scheme.

The Science Research Council, through its boards and subcommittees which allocate grants among the applicants (30 of whom are now being considered), hopes to ease stagnation in research areas that it feels deserve encouragement. Awards have been given to Professors D. H. Everett (physical chemistry, Bristol), J. G. Powles (physics, Kent), M. Symons (chemistry, Leicester) and R. Butterfield (civil engineering, Southampton).

Philip Campbell

Authority more critical

The United Kingdom Atomic Energy Authority made a forceful response last week to the critical report of the House of Commons Select Committee on Energy (see *Nature* 19 February, p.621) Dr Walter Marshall, the bulky and voluble Welshman who succeeded Sir John Hill as chairman at the weekend, was quickly in action with his account of where the select committee had gone wrong.

The committee's wrath was directed chiefly at the Central Electricity Generating Board, but it also asked that the authority's role in the development of



No-doubt Marshall

nuclear power in Britain should be restricted to research on long-term projects (fast reactors and fusion devices) and others where interested parties chose to commission work.

Marshall argues that this conclusion is mistaken. Thus he justifies the authority's work on the safety of pressurized water reactors (now costing £10 million a year) on the grounds that the authority is more independent than the would-be builders of the plant, the

generating board, and less likely to continue indefinitely in the field than, say, the Nuclear Installations Inspectorate.

On the select committee's opinion that the authority should not be the public shareholder in the National Nuclear Corporation, the publicly supported construction consortium, Marshall says that the only effect of such a change would be to replace him by a civil servant as a director of the corporation. At present, he says, the authority's representation is the only source of independent criticism on the board.

The recommendation that the authority should quickly make an assessment of the Canadian CANDU heavy-water reactor system is similarly unwelcome. Marshall says that the committee has underestimated the difficulty of adapting even well-established reactor systems to British safety regulations, and estimates that a proper assessment would require two years of hard work. He points out that the select committee overlooked the authority's role in the development and management of the nuclear fuel cycle.

Dr Marshall's succession as chairman of the authority — he has been waiting in the wings for several years — presages a change of style. He is both outspoken and ebullient. He has his roots in the research establishment at Harwell (where he will keep an office). Last week he was saying that there will be no cause for changing the role of the authority until fast reactors are commercial realities some time in the next century. But he plans that the authority should become more skilled at explaining what it is about.

Community research

Project sharing?

Brussels

The European Parliament has now called for more community research. This arose at a meeting between the Parliament's Science and Energy Committee, the Dutch Minister for Science and Technology, Anton van Trier, and Dr Guenter Schuster, the director general for research, science and education of the European commission.

Mr van Trier pointed out that between 1970 and 1980, Community expenditure on research and development had increased by 18 per cent, but that out of the 20,000 million European Units of Account (EUA) (1 EUA = £0.56) which the member states spent on their energy and research programmes in 1980, only 1.5 per cent was spent jointly. The president of the committee, Hanna Walz, while endorsing Mr Van Trier's affirmation of the significance of Community research, pointed out that there is a considerable gap between the declared intentions of the Council of Ministers and its actions.

Other members of the committee thought that the time was ripe to set in motion a "real Community research policy", since major areas could not be adequately tackled by each country on its own. Dr Schuster said that Community research would continue to concentrate on specific areas, particularly nuclear power, energy and environment, and pointed out that the Community contribution in these areas is 30 per cent, 10 per cent and 20 per cent respectively of total Community spending.

Mr van Trier and the committee agreed that a European market should be created for micro-electronic components and products, but that coordinating national programmes would only be feasible for large projects. The companies themselves must establish relations in order to coordinate the proposed research programmes.

Jasper Becker

US Cabinet appointments

Conservatives in

Washington

One of the few bright spots for US environmentalists in the Cabinet appointments has been Mr Reagan's nomination of Mr James Buckley, a member of the US Senate from 1971 to 1977, as Under-Secretary of State for National Security, Science and Technology. If confirmed by the Senate, Mr Buckley will be directly responsible for the State Department's Bureau of Oceans and International Environmental and Scientific Affairs. Under a reorganization plan being discussed in the White House, the bureau may also be given responsibility for nuclear non-proliferation policy.

Mr Buckley is a conservative Republican. He voted against the War Powers Act giving Congress greater control over presidential decisions, and also opposed the first Strategic Arms Limitation Treaty (SALT), because it limited the United States to the construction of only two anti-ballistic missile sites — which were never built.

As a member of the Senate's environmental pollution subcommittee and its environmental science and technology subcommittee, Mr Buckley helped to forge much of the stringent environmental legislation of the 1970s. He clearly intends

to take a close personal interest in environmental issues in his new post. High on his agenda is discussion with Canada of the effects of acid rain from the north-east states. Another issue is international attempts to prevent the extinction of endangered species; Mr Buckley told his nomination hearing in the Senate last week that "as a friend of the snail darter [whose potential extinction was used to hold up a federal dam project] I look forward to extending my responsibilities".

One potential dispute has already been headed off by the State Department. In its preliminary proposals for budget cuts, the Office of Management and Budget had suggested eliminating the \$10 million contributed by the United States to the United Nations Environment Programme (UNEP). This — almost a third of the agency's budget — would have devastated UNEP's future programmes, but was successfully resisted by Mr Haig, and a contribution of \$7.2 million is being proposed. In his Senate hearing, Mr Buckley said this would be "foolhardy".

Nuclear non-proliferation policy will be more difficult to deal with but will be high on the new Administration's agenda, since India is already preparing to make a further application for the export of enriched uranium to its Tarapur nuclear plant. Mr Buckley told the Senate Foreign Relations Committee that the Reagan Administration was completely reviewing non-proliferation strategy.

Mr Buckley, who ran for the Senate in Connecticut, had been tipped as the new administrator of the Environmental Protection Agency. In fact, the White House announced last week the nomination of a Colorado attorney, Mrs Anne Gorsuch, a close political ally of Interior Secretary James Watt, to head the agency. Both nominations have generated protests from environmentalists.

David Dickson

UK medical research

Money to spend

A £350,000 windfall has landed in the hands of the British Medical Research Council's Laboratory of Molecular Laboratory, Cambridge. The money was donated quite unexpectedly by Mr Thomas Usher, chairman of the Canadian company, Dextran. Since Mr Usher first approached the laboratory last May, a fund has been set up to support research fellows and students over the next seven years.

The offer of money comes with no strings attached, and the company had previously had only brief contact with the laboratory — many years ago Dr Max Perutz gave Mr Usher some advice.

The staff of the laboratory has agreed with Mr Usher that the money should be used to support research staff, the most serious hole left by cuts in the Medical

Research Council's budget. The resulting fund will provide £50,000 a year for one to three-year fellowships, short fellowships for visiting scientists and studentships over the next seven years.

The scheme is to get under way in earnest next October. The trustees of the fund — Drs Sydney Brenner, director of the laboratory, Frederick Sanger, John Gurdon, Hugh Huxley and Aaron Klug — will decide on the next recipients in June.

Judy Redfearn

High-energy physics

China backs off

Washington

Economic problems have forced the People's Republic of China to postpone construction of a 50 GeV particle accelerator scheduled to be built near the Ming Tombs, north-west of Peking.

The accelerator, to have been built with the assistance of the US Department of Energy, was central to the agreement between China and the United States signed by President Carter and Vice-Premier Deng Xiao Ping in Washington two years ago.

US official who visited China last month were told that although construction was being deferred as part of a major cutback in capital construction projects, the delay did not imply a reduced commitment to research and training in advanced science and technology. The officials feel, however, that a "softening" in China's previous commitment to high-energy physics is inevitable.

Under the agreement, the Department of Energy would have carried out much of the construction of the components of the accelerator, receiving purchase orders over a period of five years of between \$100 and \$200 million for work that would have been carried out at the national energy laboratories. Subsequently the terms were changed so that the Chinese would carry out most of the design and construction work, and the United States would only provide basic training and advice.

The Chinese have also told the National Aeronautics and Space Administration that they will have to postpone the planned purchase of a new US-built telecommunications satellite system, another major component of the science and technology agreement. Four major contractors had already discussed with the Chinese the specifications of such a system, and were preparing bids. In a letter, the head of the Chinese academy of space technology, Dr Ran Xin-Mon, has now said that China was being forced to reconsider the whole of its space programme in the light of its economic situation.

China is, however, continuing negotiations over the purchase of equipment to receive geophysical information from the LANDSAT remote sensing satellite. It has

also said that it intends to reschedule the satellite deal when funds are available.

According to Representative Donald Fugua, chairman of the House of Representatives Science and Technology Committee, who headed the delegation to Peking, China has decided that it will have to cut out about half of its ambitious construction plans in science and technology — including plans for a new steel mill to have been built outside Shanghai. But Chinese officials have stressed that other activities, such as scientific exchanges and research programmes will not be affected.

David Dickson

Soviet space research

Signs of strain

The planned Franco-Soviet mission to Halley's comet in 1986 turns out to involve a substantial cutback for the planned joint mission to Venus in 1984. This development, first indicated last summer, was finally confirmed two weeks ago in Paris. Two of the original four Venus probes have now been assigned to Halley, so that the French have had to abandon plans for a large balloon which would have analysed the atmosphere of Venus. Although the reduced Venus mission could still have accommodated a smaller balloon, the consequent problems of high-temperature electronics could not be solved in time.

According to M. Hubert Curien, president of the Centre National d'Etudes Spatiales, the French side has few regrets. The new programme, he said, is "very full and very attractive". Nevertheless, the final announcement of the scaled-down Venus mission was a reminder that the resources of the Soviet space programme, though vast, are not infinite. It came only a few days after *Pravda* had published two long articles of no great topicality clearly intended to present the Soviet space effort in a favourable light.

One dealt with the detection of iron in the lunar regolith samples recorded by the Luna-16 probe in 1970 and one with the seventieth anniversary of the birth of the late Mstyslav Keldysh, whom Khrushchev brought out of the field of military rocketry to lead the academic space programme.

The question of the scale of Soviet space research will arise this week, when the twenty-sixth congress of the Communist party of the Soviet Union will be required to approve the basic guidelines for the next Five-Year Plan. As with the two previous plans, these will include a commitment to space exploration "in the interests of the national economy". This is an empty formula for deep-space research, although satellite photography is playing an increasing part in a number of aspects of Soviet planning, from fish-spotting to geological surveying. The costs of the programme are, however, never published.

Hints that Soviet space spending may be

subject to increasing financial constraints have, however, been dropped in recent months. There seem to be no further plans for Comecon participation in manned flights after Mongolia and Romania have put a cosmonaut in orbit. Soviet planners have so far failed to respond to Bulgarian hints that, as their cosmonaut, Georgi Ivanov, failed to complete his mission (his Soyuz transport craft could not link up with the Salyut station), Bulgaria should be allowed another turn, especially in its 1,300th year of statehood. Instead, Bulgaria has been promised two unmanned probes instead of the original one.

The Soviet commitment to Comecon participation in space research nevertheless continues. A new scientific cooperation programme with Poland, announced last month, put special emphasis on space research. Poland, the homeland of Copernicus, may have a special place in Soviet space planning but the Soviet Union's contribution to Comecon collaboration is substantial. It pays the total cost of the launching and ground control. The participating Comecon partner simply has to pay for its own apparatus and the associated data processing — a privilege also extended to the French.

Vera Rich

Science and government

Lords look now

The House of Lords Select Committee on Science and Technology has taken the unusual step of making a public appeal for opinions on the subject of its latest inquiry — science and government. The inquiry's chairman will be Lord Sheffield. Lord Todd, who is thought to have instigated the inquiry, and who was expected to take the chair, seems to have stepped down in the belief that his strong views can be better aired from the body of the committee.

Central to the inquiry will be the need for a chief scientific adviser to the government and the success of the Rothschild customer-contractor principle. The chief scientific adviser's post was abandoned in 1974 after the Rothschild report recommended that individual government departments should take more responsibility for seeking advice and commissioning research. The system of departmental chief scientists that resulted was intended to enable government departments (the "customers") to commission research within the research councils (the "contractors") with money transferred to them from research council budgets.

Although the principle has worked well in some departments, it has been disastrous in others. The Department of Health and Social Security has acknowledged that it cannot place contracts for biomedical research, while the Ministry of Agriculture, Fisheries and Food is considering a proposal to abolish the post of departmental chief scientist as part of its changing

relationship with the Agricultural Research Council (*Nature* 1/8 January, p.2).

The committee started taking oral evidence yesterday (25 February) from Sir Ian Bancroft, head of the home civil service. Next on the list are Lord Trend, former Secretary to the Cabinet and author of the 1964 report on the organization of civil science, and Sir Hermann Bondi, chairman of the Natural Environment Research Council and a former chief scientist at the Ministry of Defence and Department of Energy. Those wishing to submit evidence should write to the Clerk of the Select Committee on Science and Technology, Committee Office, House of Lords, London SW1 by 31 March 1981.

Judy Redfearn

Questions to answer

A Machinery of government

(1) Should scientific advice and/or research procurement be a distinct function of government separate from the existing departmental structure?

(2) How successful is the system of departmental chief scientists in procuring advice, managing research and influencing policy?

(3) How well supported are ministers when judging scientific priorities in decision making, particularly if government departments are not in agreement?

(4) How far is the scientific advice sought by government geared to supporting predetermined objectives?

B Finance

(1) How satisfactory is the division of financial responsibility between the research councils (as a group) and government departments funding research on the customer-contractor principle?

(2) Is any research which could be of real value to government being neglected for lack of identified customers or because it is peripheral to the interest of several customers; if so, what changes could rectify this?

(3) Are any changes in research budgets desirable?

C Machinery of science

(1) How adequate are the channels of communication from the scientific community to government, and vice versa?

(2) Is there satisfactory contact between those administering science in higher education, industry, the research councils and government?

(3) How could statutory procedures for consultation by government in scientific matters be improved?

(4) Are existing sources of advice adequate to ensure that the United Kingdom gains all it can from EEC and international research programmes?

D Scientific manpower

What manpower constraints are there on the provision of scientific advice to government?

CORRESPONDENCE

Genes and race

SIR — I write in reply to Dr Steven Rose's letter (*Nature* 22 January, p.335), which drew attention to the fact that a right-wing journal, *New Nation*, has quoted me, together with other evolutionary biologists, in support of their view that our genetic constitution makes it impossible for us to live in a racially integrated society. I welcome the opportunity to say that there is nothing in modern evolutionary biology which leads to this conclusion.

JOHN MAYNARD SMITH

University of Sussex,
Falmer, UK

Museum debate

SIR — I have been following the "great museum debate" in your pages with a profound sense of detached amusement. But as matters are quickly reaching a level of absurdity that may inspire me to write the 15th Gilbert and Sullivan opera, and as I am, in a sense, the focal point for Halstead's glorious uproarious misunderstanding, I suppose I should have my say.

Halstead began all this by charging that the venerable Natural History Museum is now purveying Marxist ideology by presenting cladism in its exhibition halls. The charge is based on two contentions: (1) a supposed link between the theory of punctuated equilibrium, proposed by Niles Eldredge and myself, and cladistic philosophies of classification; and (2) an argument, simply silly beyond words, that punctuated equilibrium, because it advocates rapid changes in evolution, is a Marxist plot. For the first, there is no necessary link unless I am an inconsistent fool; for I, the co-author of punctuated equilibrium, am not a cladist (and Eldredge, by the way, is not a Marxist, whatever that label means, as if it mattered). Under cladism, branching events may proceed as slowly as the imperceptible phyletic transitions advocated by the old school. Punctuated equilibrium does accept branching as the primary mode of evolution, but it is, fundamentally, a theory about the characteristic rate of such branching — an issue which cladism does not address.

For Halstead's second charge, I did not develop the theory of punctuated equilibrium as part of a sinister plot to foment world revolution, but rather as an attempt to resolve the oldest empirical dilemma impeding an integration of palaeontology into modern evolutionary thought: the phenomena of stasis within successful fossil species, and abrupt replacement by descendants. I did briefly discuss the congeniality of punctuational change and Marxist thought (*Paleobiology*, 1977, p.145) but only to illustrate that all science, as historians know so well and scientists hate to admit, is socially embedded. I couldn't very well charge that gradualists reflected the politics of their time and then claim that I had discovered unsullied truth. But surely Halstead, who has done some statistics in his day, knows that correlation is not cause. If I may make a serious point: I grew up frightened in a leftist household during the worst days of McCarthyism in

America; and I know that what seems peripheral or cranky today can become a weapon tomorrow (consider the current creationist surge in America). May we avoid red-baiting; it may not always be harmless.

I saw the cladistic exhibits last December. I did not care for them. I found them one-sided and simplistic, but surely not evil or nefarious. I also felt, as a Victorian aficionado who pays homage to St Pancras on every visit to London, that most of the newer exhibits are working against, rather than with, the magnificent interior that houses them. But I would not envelop these complaints in ideological hyperbole; Halstead has said enough.

STEPHEN JAY GOULD

Museum of Comparative Zoology,
Harvard University,
Cambridge, Massachusetts, USA

Last word?

SIR — It is a little late in the day for Stephen Gould to try and come the innocent. If he did not want "Halstead's glorious uproarious misunderstanding" to get under way, he should have avoided dragging Engels on to his side in the first place¹. Mind you, even this might not have worked, for the "political" implications of punctuated equilibria have not gone unremarked, as some of the recent correspondence has made manifest.

I have taken Engels and Lenin as my main sources on dialectical materialism, which I have sought to apply in the conduct of my own researches. There is only one point, but this is fundamental, at which I would part company with Marxism and that is the nature of qualitative changes, which I see not as sudden leaps but as gradual in the tradition of Charles Darwin. I have recently been non-plussed to learn from Stephen Gould that "many orthodox Marxists have been quite content with Darwinian gradualism" (ref. 2). This is equivalent to someone, who insists that Christ was a myth, being considered an orthodox Christian. But perhaps in the United States, I would be deemed an orthodox Marxist!

When it comes to the cladists with their punctuated tendencies, we run into a perfectly ludicrous source of semantic confusion. If, as some of the correspondents have insisted, cladistics is concerned only with pattern and not process, then obviously there is no point in arguing further, because I am concerned primarily with process. I wrote about the kind of classical Hennigian cladistics actually being presented in the public galleries of the British Museum (Natural History) and clearly explained in their accompanying booklets, and not the new transformed variety of Patterson³ and others.

Tempting though it may be, I am sufficiently modest to decline the mantle of oracle proffered by Rosen⁴ with regard to the origin of *Homo sapiens* from *Homo erectus*. I claim no special insight in these matters but merely reported the considered and published consensus of the staff of the British Museum's own Sub-Department of Anthropology. Wood⁵ has drawn attention to the "dubious academic practice" of ignoring "uncomfortable" evidence — the scandal of

this is that it was deliberate, involving, as it did, the overruling of the museum's own experts. Critical scientific evidence is being deliberately withheld from the public who are, in consequence, being seriously misled as to the nature of "Man's place in evolution". All is apparently being subjugated to a chosen dogma. I have been roundly abused for implying that a far left political connection might be involved, but the present dogmatic policies seem to bear its unmistakable stamp.

The British Museum (Natural History), London, is a major public scientific institution in this country and as such should be accountable to the public. It is surely reasonable to expect the Director to answer the charges that have been levelled — a continued "dignified silence" in the present circumstances is simply not good enough.

L. B. HALSTEAD

Departments of Geology and Zoology,
University of Reading, UK

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Badger controversy

SIR — On 11 December 1980 the Mammal Society published a letter in *Nature* drawing attention to some of the many anomalies in the Zuckerman report. The reasons we started an open discussion of the report are obvious. Badger gassing started in 1975; nearly six years later, after the expenditure of a great deal of time, money and effort, it is our belief that there is no unequivocal evidence that the present gassing policy is likely to produce a long-term solution to the problem. We suggested that the Zuckerman report is one-sided in its interpretation of the evidence, and that the conclusions and recommendations in the report are too categorical and do not take sufficient account of the complexity of the problem.

Following our original letter, three letters have been published in *Nature*. Dr Plowright (1/8 January 1981, p.8) presented no new information, nor did he answer any of the points we raised, and so his letter will not be discussed further. Dr Yates (22 January 1981, p.218) questioned a graph sent to Lord Zuckerman for his comments. This graph was simply intended to show that the rate of decline of TB was similar in the South West to that elsewhere in England. That the incidence of TB was different in the two areas was never disputed. Dr Yates published an alternative graph, which was simply another presentation of the same data; it showed that the incidence of TB varies in different parts of the South West. That is the very point we stressed in our original letter. Dr Yates' graph also showed that there was a decline in TB in all the regions sampled, irrespective of whether badger gassing was carried out in that area. That is the only point our original graph was designed to demonstrate.

In fact Dr Yates' graph has highlighted the

Continued on page 831

NEWS AND VIEWS

Models of speciation — the evidence from *Drosophila*

from J.S. Jones

IF Lewontin's (1974)¹ claim that "we know almost nothing of the genetic changes that occur in species formation" is still true then we must by now know it in remarkable detail, for the literature of evolutionary biology has been filled with speculations, observations and experiments on this very subject for the last five years. There are two models of the origin of species. In one of these² the division of a population into two genetically incompatible entities is seen as a by-product of the gradual adaptation of its geographically isolated parts to different selection pressures. Hybrids between the evolving populations are relatively unfit because they have accumulated different alleles with individually minor effects. Reproductive isolation thus arises at first from the incompatibility of separately developing gene pools; should these pools later come into contact, selection may lead to the evolution of behavioural or ecological barriers which reduce the frequency of hybrid matings. The origin of a new species is the result, writ large, of the familiar process of natural selection within an existing species.

A second model — that speciation is brought about by a 'genetic revolution' — has recently returned to prominence³⁻⁵. This theory places less weight on selection, and emphasizes the importance of random processes in the origin of taxonomic groups. A small and isolated population of an originally widespread and highly variable species will, it is suggested, undergo major genetic changes because of the chance loss of alleles in the small founding sample, the increased frequency of homozygotes caused by inbreeding in the early generations and the resulting disruption of interacting complexes of genes and of developmental processes under genetic control. The new isolate may move into previously unexploited ecological niches and undergo an enormous increase in numbers (the 'population flush'³) with a consequent relaxation of selection against deviant individuals which will further favour the success of the genetic revolution. Rapid fluctuations in population size and random gene frequency changes in these early generations will quickly lead to the evolution of reproductive incompatibility with the parental population. According to

this view, isolation causes speciation, rather than sometimes allowing it to take place as an incidental result of slow adaptive evolution.

Recently, some palaeontologists have claimed that this model of speciation can explain the apparent gaps in the fossil record⁶. Rather than accepting that the gaps reflect the record's incompleteness, they claim that they are the result of a pattern of bursts of rapid speciation interspersed with long periods of evolutionary stability. Only rarely will members of the small speciating population be fossilized, so that the lineage will show what are known as 'punctuated equilibria' — a long series of unaltered fossil forms followed by a rapid shift to a new type. Species are therefore discrete units not only in space, but also in time.

Some biologists have interpreted this view of evolution as an attack on the fundamentals of Darwinism and a bitter dispute is still taking place. However, the problem can be approached experimentally using *Drosophila*. There is now good evidence that some populations may indeed contain hidden genetic variation sufficient to cause 'instant speciation' and that rapid fluctuations in population size can sometimes produce reorganizations sufficient to lead to reproductive isolation within a very few generations.

Drosophila mercatorum is normally sexual, but in the laboratory a female can produce parthenogenetic clones. It is possible to freeze the recombinant offspring of a single heterozygous female into a number of diverse clones, and by comparing these to estimate the amount of hidden genetic variation in the original population. Templeton and his colleagues^{7,8} find that such clones differ from each other in morphology, in behaviour and in aspects of fitness sufficient to cause rapid changes in their frequency when competing in a population cage. Most important, when females from some of the derived clones are tested for mating potential with males from their ancestral sexual population, they show strong reproductive isolation from them. What is effectively a new species of *Drosophila* has therefore arisen as a result of reorganizations of the genome of a population within a few generations.

Sexual populations may also undergo genomic transformations of this kind. A population of *D. pseudoobscura* was placed in a large cage and allowed to

expand rapidly in numbers⁹. At the peak of the population flush, 8 single pairs were chosen to start 8 new populations, each of which grew to a large size. These populations were put through three further cycles of reduction and expansion. Inter-population tests of mating preference showed that in a third of the derived cages there was a significant tendency for individuals to prefer to mate with others from their own population. Reproductive isolation was the result of the demographic (and associated genetic) changes within the populations, as control populations isolated from each other without undergoing rapid changes in population size did not mate preferentially.

Population flushes in nature are most likely to occur in organisms which disperse easily, have short generation times and live in regions where new and unexploited habitats become available. The *Drosophila* of Hawaii are a good example of such a group. The Hawaiian archipelago came into being some 40 million years ago, and the youngest island is about 700,000 years old. Nearly a third of all known *Drosophila* species occur only in Hawaii, suggesting that speciation has been extremely rapid. Carson¹⁰ suggests that this reflects a history of founder events, population flushes, and the formation of new gene combinations as flies blew from one island to that formed next in the volcanic chain. On the basis of chromosome phylogeny he estimates that as few as 22 inter-island colonizations (many of which may have involved just one fertilized female) over only 6 million years will account for speciation in the best known group of Hawaiian *Drosophila*. The rapidity of speciation is a result of random events and demographic changes leading to reproductive isolation, rather than of slow adaptive evolution on each island.

The patterns of reproductive isolation between flies from different islands support this view¹¹. In *Drosophila* the female selects the mate; a courting male must produce the correct sequence of behaviours before being accepted, but a male will attempt to mate with almost any available female. Reproductive isolation between species of Hawaiian *Drosophila* from different islands is often asymmetrical. A female from a new species on a young island will accept a male from the ancestral population, but a male of the new species cannot successfully court a female of the ancestral species. This is evidence for

J.S. Jones is in the Department of Genetics and Biometry, University College London.

rapid speciation in a founding population; the males of the derived species lost, by chance, in their small founding population some of the behavioural repertoire which made them acceptable to ancestral females, but the ancestral males retain all the genes for the behaviour needed to stimulate females of both species.

This process has been simulated in the laboratory by an inadvertent experiment of the kind familiar to *Drosophila* geneticists¹². A stock of Hawaiian *D. silvestris* underwent at least two population crashes (perhaps to a single fertilized female) during its stay in the laboratory. The derived stock showed strong behavioural isolation from freshly collected *D. silvestris* from the same location. Wild males successfully courted derived females, but derived males could not stimulate females from the ancestral population. Asymmetrical reproductive isolation did not develop as a by-product of slow adaptive evolution but resulted from extreme demographic changes.

As species are, by definition, genetically isolated from each other, it might seem impossible to investigate the genetics of reproductive isolation by carrying out breeding experiments. This apparent contradiction has been overcome in the Hawaiian *Drosophila*¹³. *D. silvestris* and *D. heteroneura* are known to be closely related, but differ markedly from each other in head shape. This is an effective behavioural isolating mechanism in the wild. It is possible to make fully fertile crosses between these species in the laboratory, and genetic analysis shows that the differences in head shape are due to a single sex-linked locus which influences the phenotypic expression of about 10 interacting autosomal loci. The genetic basis of each species' integrity thus depends on rather few loci — a result consistent with theories emphasizing the importance of random events in the origin of species.

Although these views of speciation as a 'revolutionary' process are now receiving much attention, there is also evidence strongly supporting the view that many species originate through the slow evolutionary accumulation of small variations. Recent theoretical work by Lande¹⁴ shows just how effective selection acting on many genes with individually small effects can be in leading to the divergence of large populations. His predictions are supported by many experiments in which individual *Drosophila* prefer to mate with others from the same natural population¹⁵ or in which the offspring of inter-population crosses are less fit than those whose parents arise from the same population¹⁶. Laboratory stocks subjected to different temperature and humidity stresses accumulate over a period of time genetic differences great enough to lead to mating preferences or hybrid inviability¹⁷. Reproductive isolation has also been found to occur between populations of *Drosophila* and of house flies selected for high or low activity, for different bristle number and for DDT resistance¹⁸⁻²¹.

In summary, there is convincing evidence for both models of the origin of species. In some lineages, speciation may result mainly from natural selection acting on genes with small effects, while in others it probably results from the disruption of a genetic system following the random colonization of a new environment by a small population. The two mechanisms are not mutually exclusive and within one genus both may be important. In *D. mercatorum* (where experiments with derived clones show that natural popu-

lations contain the genetic potential for very rapid speciation) geographically separated populations have differences in their behaviour which have arisen by the accumulation of many genes with small effects — and which are sufficient to cause considerable reproductive isolation²². In the Hawaiian *Drosophila*, also, where evidence for rapid speciation as a result of random effects is so strong, some populations from the same island have undergone considerable divergence which is not a result of founder events²³. Any complete theory of the origin of species must recognize — as did Darwin himself — the importance of the joint action of random processes and of natural selection.

Models of speciation have recently become entangled with claims that the 'punctuated equilibrium' theory of evolution favoured by those responsible for the dinosaur gallery in the Natural History Museum reflects that of the evolution of society held by Karl Marx²⁴. Whatever the scientific merit of an argument couched in terms of Dinosaurs versus Democracy, it is worth considering the attributes of a lineage liable to undergo rapid speciation by genetic revolution^{4,5}. They include an abundant and freely interbreeding ancestral population, high individual mobility, and rapid reproduction. Although the *Drosophila* of oceanic islands fit these criteria well, it is hard to imagine any group less able to satisfy them than the dinosaurs. In particular, the idea of a windblown propagule of a pregnant female *Brontosaurus* is enough to strain the imagination of even a palaeontologist! □



100 years ago

MIGRATION OF THE WAGTAIL

I fear I may be attempting to trespass too frequently on the columns of *Nature* recently, but the paper on the subject of wagtails taking a passage on the backs of cranes in a long flight, resembles so much a somewhat similar story told and believed in by the Indians in several parts of North America, that I venture to send you an account of it.

All the Indians (Maskegon Crees) round the south-western part of Hudson's Bay, assert that a small bird of the Fringillidae tribe takes a passage northward in the spring on the back of the Canada goose (*A. Canadensis*), which reaches the shores of Hudson's Bay about the last week of April. They say that they have often seen little birds fly away from geese when the latter have been shot or shot at.

An intelligent, truthful, and educated Indian named George Rivers, who was very frequently my shooting companion for some years, assured me that he had witnessed this, and I believe I once saw it occur.

It is only the Canada goose that these little

migrants use as an aerial conveyance, and certainly they both arrive at the same date, which is a week or two earlier than the other kinds of geese (*A. hyperboreus* and *albifrons*) make their appearance. I knew the little bird well, but it is so long ago that I have forgotten the name.

The Indians on the shores of Athabasca and Great Slave Lakes, both great resorts of wild geese, tell a similar story. If a fabrication, I do not see why it should be invented about the Canada goose only, and not about other species which are equally numerous.

All the Coast Indians of Hudson's Bay devote a month or more every spring to wild fowl (chiefly geese) shooting. As soon as the geese begin to arrive, the Indian constructs a concealment of willows and grass, usually near a pool of open water, at the edge of which he sets up decoys. When geese are seen approaching (usually flying at a great height) the Indian imitates their call, and the geese on seeing the decoys circle round, gradually coming lower down until within shot, when they are fired at. It is from these high-flying geese that the small birds are seen to come.

If the geese are flying low it is a pretty sure indication that they have already rested on the ground somewhere near, after their long flight, when of course their tiny passengers would have alighted.

JOHN RAE

Royal Institution, February 26
from *Nature* 23, 3 March, 411, 1880

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Last supper of two Indian Triassic reptiles?

from Barry Cox

DURING the winter of 1965–66, Indian palaeontologists found a pair of skeletons of the crocodile-like phytosaurian reptiles in the late Triassic rocks of the Maleri Formation in the Pranhita-Godavari Valley, Andhra Pradesh, central India. They were found so closely associated that they soon became known as 'the loving couple'. During the removal of the soft, clay-like matrix from their skeletons, it soon became clear that they had also eaten a similar last meal, for within the rib cage of each there appeared the skeleton of a smaller reptile.

These two skeletons have now been described by Chatterjee (*Phil. Trans. R. Soc. B* **291**, 163; 1980) as a new genus, *Malerisaurus*. It belongs to a group of still poorly known and poorly understood Permo-Triassic lizard-like reptiles that includes the ancestors of the lizards themselves. *Malerisaurus* was of considerable size, some 1.3 m long, and it is notable for its rather large hind-limbs. The length of the fore-limb is only 59 per cent of the length of the hind-limb, and the latter is 74 per cent of the length of the

Barry Cox is Professor of Zoology at Kings' College, London.



Life restorations of two specimens of *Malerisaurus*, showing quadrupedal and bipedal gaits.

trunk. Proportions of this kind are found today in a number of facultatively bipedal lizards, such as the frilled lizard *Chlamydosaurus*, and the life restorations of the two specimens that illustrate Chatterjee's paper show them in two alternative poses. The dentition of *Malerisaurus* suggests that it fed on the general range of invertebrates that is described as an 'insectivorous diet'.

The sedimentology of the Maleri deposits suggests that the environment of this part of India during the late Triassic

was probably very similar to that found there today, with a subtropical monsoon climate. *Malerisaurus* therefore probably lived in a densely forested, lowland environment with lakes or flood-plains.

The Maleri fauna also contains a variety of other reptiles, as well as the large aquatic amphibian *Metoposaurus*. All of these belong to groups that are widely known throughout the Triassic world, and attest to the ease of dispersal through that ancient Pangaea world-continent.

Is the Universe expanding?

from David A. Hanes

THE DISCOVERY by Edwin Hubble¹ that the Universe is not static, but an evolving entity, is surely the most important finding in extragalactic astronomy this century. Few astronomers would contest the view that the Universe is in a state of general smooth expansion. However, recent provocative statistical studies by J. F. Nicoll, D. Johnson, I. E. Segal and W. Segal² of the relationship between the redshifts of moderately nearby galaxies and their apparent brightnesses have brought this simple yet profound picture under attack.

In a general expansion, the recession velocity of a galaxy (or empirically its Doppler redshift) should be directly proportional to the distance of that galaxy. Nicoll *et al.* claim they can reject such a relationship; rather, their findings imply a quadratic relationship between redshift and distance for a moderately nearby sample of galaxies (those with recession velocities less than 5,000 km s⁻¹). Such a discovery is consistent with the predictions of I.E. Segal's³ alternative world model, the chronometric cosmology, and at

variance with the predictions of Einsteinian General Relativity. At the very least, these statistical demonstrations demand explanations in terms of unsuspected selection effects, subtly biased samples, or local anisotropies or inhomogeneities; and, should convincing explanations be lacking, they may suggest that the usual cosmological descriptions of the Universe are inadequate.

Edwin Hubble's original data suggested a linear relationship between velocity (inferred from redshifts) and distance (inferred from apparent luminosities) for galaxies. This relationship has an appealing simplicity, for in a universe in which we are in no preferred position and where there are no preferred directions — that is, in a universe where the 'cosmological principle' holds — it can be shown that such uniform expansion (or contraction) is the only permitted large-scale motion. The cosmological principle is justified both philosophically and empirically: the former in the neo-Copernican sense that we must assume that we are in no way singled out as special observers of the cosmos; and the latter in that galaxy counts and the isotropy of the cosmic background radiation suggest that the Universe is indeed

homogeneous and isotropic on sufficiently large scales⁴. There are other reasons for the faith of astronomers in the expansion hypothesis: the expansion is associated with a characteristic time (which may naively be thought of as the time since the Universe was in a state of infinite density) which is closely comparable with the ages of the oldest known stars; and the discovery⁵ of the predicted⁶ cosmic microwave background strongly suggests that the Universe has evolved from a hotter dense state. Moreover, the Hubble expansion finds a natural theoretical framework — that of Einsteinian General Relativity — which in fact predates⁷ the discovery of the expansion and which has been amply confirmed in the limited tests possible to date⁸.

For all these reasons astronomers will be unmoved, except by unequivocal tests, to adopt alternative cosmological descriptions of the Universe. Segal's chronometric cosmology is one such description. It has been characterized⁹ as a 'gravitational tired light' theory; that is, one in which observed redshifts for remote galaxies arise through the loss of energy by photons during their propagation rather than because of any systematic motions. In Segal's

David A. Hanes is at the Anglo-Australian Observatory in New South Wales, Australia.

formulation, the chronometric theory reproduces many of the observational attributes of the cosmos, such as the universal background radiation; and the telling coincidence of inferred expansion age with stellar age is not a problem¹⁰, although arbitrarily old galaxies may abound. Astronomers — indeed, all scientists — are reluctant to adopt purely *ad hoc* theories, those which follow rather than predict fundamental discoveries and which explain later observation by judicious manipulation of the theory, and this kind of criticism has been levelled at the now largely discredited Steady State cosmologies. However, Segal's chronometric cosmology does make a crucial prediction relating to the behaviour of the redshift–apparent magnitude relation for systems of moderate redshift (inferred velocities $\leq 5,000$ km s⁻¹): the observed relation should be quadratic.

The statistical study of such nearby systems is plagued by a variety of important selection effects. Complete redshift determinations are available only for the brightest few hundred galaxies. At fainter levels, galaxies of special interest — those in rich clusters, or those associated with bright galaxies — may provide additional data. The galaxy luminosity function is broad, and an apparently bright galaxy with well determined redshift may be a remote very luminous object, while nearby fainter galaxies go unstudied; thus volume-limited samples are not to be hoped for. Perhaps most important, the local dynamics enter into any analysis: the corrections for solar motion around our own galactic centre, the peculiar motions of single galaxies and the dispersion of velocities because of interactions in clusters of galaxies are all relatively large compared with Hubble expansion rate for the nearby systems: the motion of our own local group of galaxies, which appears to be falling into the rich Virgo cluster¹¹, may introduce systematic effects; and other anisotropies may be present.

Deviations from the simple Hubble law have in fact been noted in the past. Hubble¹³ himself recognized a degree of

nonlinearity in his data and concluded that the gravitational deceleration of the expansion was the cause. Vaucouleurs¹³ interpreted similar findings in terms of local departures from the Hubble flow caused by the existence of a Local Supercluster of galaxies. Other anisotropic features in the flow, the so-called Rubin–Ford effect, have received various suggested explanations¹⁴. The lesson to be drawn is that the purely astronomical interpretation of such effects is not at all clearcut, even in the simplifying context of a general underlying expansion.

Statistical tests must obviate these problems by the careful definition of completely unbiased samples unaffected by subtle selection effects. The difficulty of the exercise is reflected in the fact that R. M. Soneira¹⁵, in a careful statistical reworking of Segal's earlier analysis, found no disagreement with the expansion hypothesis but felt able to exclude the correctness of the chronometric prediction at better than the 99.999 per cent formal confidence level. However, Soneira's analysis has in turn been criticized by

Segal¹⁶ and by Nicoll and Segal¹⁷, who reach the opposite conclusion at comparable confidence levels. Their latest papers represent the most exhaustive statistical analysis to date, with the authors concluding that... "the Hubble law lacks an objective statistical foundation".

The validity of this conclusion and the resolution of the questions raised about our understanding of the large-scale structure of the cosmos may come in further statistical analyses which confirm or deny the various treatments. It may be that considerably more observational data and careful mapping of local anisotropic effects will prove essential. If the suggested nonlinearities are found to exist, most astronomers will seek explanations in terms of local deviations from the Hubble flow rather than in the wholesale rejection of the presently believed cosmological description of the Universe, with its history of successful predictions and supportive tests. The onus is upon the advocates of alternative theories to demonstrate the absolute insufficiency of any such explanations. □

Parasitology in Venezuela

from Neil Brown and Win Gutteridge

THE study of parasitic diseases has for too long suffered from the geographical separation of pure and applied research efforts. Within the afflicted countries, research has concentrated on immediate clinical and epidemiological problems while more fundamental research has been confined to Europe and North America. As a consequence, both aspects of research have suffered, one through lack of modern know-how and the other through concentration on highly selective and convenient laboratory infections. A number of countries and international funding organizations have sought to end this separation and it is encouraging to report that, in Venezuela, a recent congress* demonstrated that fundamental research in parasitology is now being actively developed alongside clinical and epidemiological aspects.

Two protozoan diseases are of primary concern in Venezuela, Leishmaniasis and Chagas' disease. *Leishmania* are intracellular parasites of 'professional' phagocytes. Infection is initiated by a flagellated promastigote form inoculated by the sand-fly (*Phlebotomus*). Promastigotes actively secrete protein, and Hernandez and colleagues (Universidad Central de Venezuela, Caracas), in association with Greenblatt's group (Hadassah Medical School, Jerusalem), reported that as much as ten per cent of all

protein synthesized is secreted. Although the secretory products have not been fully characterized, one, a glycoprotein with a high proportion of carbohydrate, has been shown by Hernandez' group to have the capacity to modify the membrane characteristics of macrophages.

Studies reported by Perez and associates (Instituto Venezolano de Investigaciones Científicas, Caracas) have delineated a number of aspects of the host response to American cutaneous leishmaniasis. In syngeneic mice the type of lesion is determined mainly, but not exclusively, by the genetic composition of the host; parasite isolates also differ somewhat in their behaviour in one host genotype. Parasite destruction was shown by Perez to be mediated in an *in vivo* assay by peritoneal cells from immune animals and immune serum; non-adherent peritoneal cells were not protective. By contrast, in genetically susceptible BALB/c mice adherent cells were not protective and were found to have suppressor activity. Relating their laboratory studies to epidemiological observations, these workers were able to show that temporary protein deficiency ablates irreversibly the capacity of resistant C57BL/6 mice to resolve an active lesion. Other factors rather than simple killing of parasites by immune serum and activated macrophages must be involved in

Neil Brown is in the Parasitology Division, National Institute for Medical Research, London and Win Gutteridge in the Biological Laboratory, University of Kent.

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*The 30th Convention of the Venezuelan Association for the Advancement of Science was held in Merida from 9 to 14 November 1980.

Leishmania immunity, however, as even in C57BL/6 mice, lesions take 5–6 months to resolve, long after these immune responses appear. Practically nothing is known in detail about parasite antigenicity, particularly the antigenicity of the surface and secretory products of amastigotes, or the changes produced in the surface structure of the phagocytes they parasitize and eventually destroy. There is an urgent need to characterize the parasite side of the interaction and the role it plays in inducing parasite-specific and possible autoimmune protective and pathological reactions.

Little more is known of the biochemistry and molecular biology of *Leishmania* except in so far as it relates to energy metabolism, purine salvage and kinetoplast DNA. Ramirez and his colleagues (Universidad Central de Venezuela, Caracas) are now, however, beginning to probe the organization of the ribosomal RNA genes. They have estimated the molecular weight of the total genome to be 1.6×10^{11} (range for other protozoa $1-6 \times 10^{11}$) and that this includes about 350 copies of ribosomal DNA. An 8-megadalton fragment of total DNA, obtained by restriction endonuclease digestion with *Bam*HI and which hybridizes with ribosomal RNA, has been inserted into plasmid pBR322 and cloned in *Escherichia coli* $\times$ 1776. It should therefore be possible in future to study the ribosomal DNA of *Leishmania* in much more detail. In a complimentary investigation, Valbuena and his colleagues (Universidad Central de Venezuela, Caracas) have begun to study messenger RNA from the organism. They have developed a method for the isolation in high yield of polyribosomes which are active in a wheat germ-derived cell-free protein synthesizing system; messenger RNA is extracted from these.

Trypanosoma cruzi, the causative agent of Chagas' disease, is also an intracellular parasite, particularly of muscle tissue of the heart and alimentary tract where it produces severe disruption of organ function. As Torrealba (Universidad de Carabobo, Valencia) pointed out, it is a zoonosis which exists from the south of the US to the northern parts of Argentina and Chile. More than 150 species of mammal are carriers and serve as potential reservoirs of infection and more than 100 species of triatomid bug, the vector of transmission, are infected. In some areas of Latin America, infection rates in man are greater than fifty per cent.

A major problem for biochemists and immunologists studying parasites is obtaining adequate amounts of material. Avila (Pan America Center for Research, and Training in Leprosy and Disease of the Tropics, Caracas), who had already reported that epimastigote forms of *T. cruzi* can be grown *in vitro* in much simpler media than those currently in use, described the successful subculture of the organism in a medium containing salts,

glucose, two growth factors and bovine liver catalase. The protein needed to be in its native configuration and was apparently not contaminated by purines, pyrimidines and growth factors. This observation suggests that the nutritional requirements of *T. cruzi* are markedly less exacting than those of other, non-endosymbiont-containing trypanosomatids. If confirmed, it will make life a lot easier for all *T. cruzi* investigators.

Piras and his colleagues (Centro Medico Docente La Trinidad, Caracas) made extensive use of probes such as lectins, microtubule disruptors and inhibitors of RNA and protein synthesis in their investigation of the mechanism of infection of fibroblastic cells by *T. cruzi*. The host cell membrane plays an active role in infection; in particular, surface glycoprotein(s) and an intact cytoskeleton must be present. Surface components of the parasite are also important; they may differ in each morphological form and some are possibly lacking in amastigotes isolated from cells since these are not infective. As low concentrations of actinomycin D and puromycin block infection

it was suggested that both parasite and host cell membrane components need to interact and function if infection is to occur.

Considerable progress has been made in understanding the metabolism of *T. cruzi*, but few studies have been made of its regulation. Urbina and his colleagues (Universidad Central de Venezuela, Caracas) reported that *T. cruzi* contains a complete glycolytic sequence and tricarboxylic acid cycle but although glucose is metabolized, there is evidence for high rates of amino acid catabolism. Further investigations demonstrated that in *T. cruzi* there is little regulation of glycolysis by sugar phosphates or tricarboxylic acids but that amino catabolism is tightly regulated by energy charge.

Basic research in parasitology, using modern techniques, is developing rapidly in Venezuela. Most of the scientists running the programmes mentioned here have, as a result of national policy, spent some years in laboratories in Europe and North America. Clearly this policy is beginning to pay dividends. □

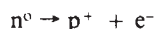
Weighing neutrinos: a small wait for a small weight?

from Frank E. Close

Do neutrinos have mass? The answer to this long-standing question may be answered within a few months if a proposal put forward by Alvaro De Rújula of CERN, Geneva is successful.

To provide the background to De Rújula's important idea it is worthwhile first recounting some of the history of neutrinos — mysterious particles produced in radioactive decays, which have no charge and (possibly) no mass, interacting so rarely with matter that they pass through the Earth as if it were empty space.

In β -radioactivity a neutron changes into a proton, and an electron is emitted to conserve electric charge:



However, the energy and momentum of the proton and the electron do not equal that of the neutron. Rather than give up the principle of energy-momentum conservation, Pauli proposed, in 1931, that a third particle was produced in the process, and that this invisible particle (the 'neutrino') carried off the residual energy and momentum. The difference between

the neutron mass and the mass of the proton plus electron would be the maximum mass that a neutrino could have and still be produced. Electrons were observed to be produced with energies up to about 1 MeV which showed that the neutrino was very light; indeed it could even have no mass at all. As no conclusive evidence for a mass has ever been found it is frequently referred to as 'massless'.

A quarter of a century passed before Cowan and Reines detected neutrinos being produced in the Savannah River Reactor in the US. These neutrinos were seen to induce the reverse reactions to those that had produced them in the first place. For their discovery they gained a case of champagne from Pauli — he had wagered that no one would ever detect so ghostly a particle!

Before turning to the ways that neutrino masses might be measured, the importance of massive neutrinos in cosmology is worth mentioning.

The density of neutrinos in the Universe should be similar to that of photons, that is at least 10^8 per nucleon. As a nucleon weighs about 10^9 eV, then neutrinos with masses even as small as 10–100 eV would provide the bulk of the Universe's mass! This would dramatically affect the expansion rate of the Universe and could

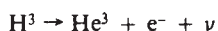
Frank E. Close is in the Theoretical Division of the Rutherford Laboratory, Didcot, UK.

even slow it to the point where it stopped and then contracted to a big crunch — a replay of the big bang but in reverse. Cosmologists claim that the presently observed expansion rate probably limits the sum of the masses of all of the three known varieties of neutrinos to less than 100 eV, maybe less than 50 eV.

There have been some indirect hints that neutrinos have masses. The flux of neutrinos impinging on the Earth from the Sun's nuclear reactions (predominantly deuteron fusion $p + p \rightarrow de + \nu$) is a factor of 3 or 4 less than that expected by astrophysicists. This 'shortfall' can be understood if neutrinos have masses (see *Is the Sun still shining?* *Nature* **284**, 507; 1980).

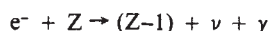
The classic way to measure the neutrino mass has been through the study of the high-energy end of the spectrum of the electron produced along with the neutrino in the β -decay. At first sight this is an obvious approach — the heavier the neutrino, the lower will be the maximum available energy for the electron. The problem is that the neutron mass is not known to better than 40 eV and so one cannot resolve the neutrino mass to less than this. However, the *shape* of the high-energy tail of the electron energy spectrum depends on the mass of the accompanying neutrino (this is a kinematic property of phase space when three particles are produced as, for example, in $n \rightarrow p + e^- + \nu$).

Recently this technique has been employed in the USSR using tritium and studying the electron energy spectrum in



This experiment claims the first non-zero mass result for ν — between 14 and 46 eV (V.A. Lubimov *et al.* *Phys. Lett.* **94B**, 266; 1980). Questions have been raised as to whether a zero mass is definitely ruled out.

In a widely attended seminar at CERN, de Rujula pointed out that there is another way that β -radioactivity produces neutrinos in a three-body final state which would be amenable to the 'shape dependence' test. Instead of producing an electron in $n \rightarrow p + e^- + \nu$ you capture an atomic electron. The nuclear capture of atomic electrons produces a continuous radiation spectrum



The photon plays the role that was previously played by the electron: measure the shape of the high-energy tail of the photon spectrum and you can infer the neutrino mass. The photon has no electrical charge and does not experience some of the scattering problems that beset charged electrons. Furthermore, detectors are available that can measure the photon energies with a precision of less than 1 eV!

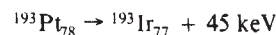
If this was the whole story then one would have been doing such experiments

long ago. The problem is that the event rate at the high-energy end of the spectrum tends to be so low that this approach is, at best, no improvement on the conventional technique. However, De Rujula noticed a feature of the radiation spectrum that could be exploited if a suitable nucleus existed and which would dramatically enhance the rate so allowing the experiment to be performed. Out of all the known isotopes it seems that Nature may have provided two that meet the crucial requirement! To explain this requirement we must start by detailing the nature of the radiation spectrum.

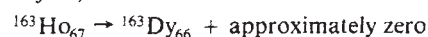
The first calculations of the intensity resulting from K-capture were made by Morrison and Schiff in 1940 (*Phys. Rev.* **58**, 24). While their calculations were in excellent agreement with the high-energy photon spectrum the observed spectra showed a considerably greater abundance of low-energy γ rays. Glauber and Martin (*Phys. Rev.* **104**, 158; 1956) showed that this enormous enhancement was due to radiative capture of electrons in L-states, the intensity peaking in the neighbourhood of the characteristic X-ray line. Thus for Fe^{55} , as an example, the peak is in the region of tens of keV while the low-intensity K capture continues all the way to the maximum available (230 keV).

If neutrinos have masses then the shape of the high-energy extreme of this distribution will be modified. Thus, although in principle we have a new way of determining the neutrino mass, the low event rate makes the method impractical. Can one find cases where the spectrum only extends for some tens of keV so that the L (or M) capture enhancement is at the high-energy end? In such circumstances the event rate will be enhanced in the very region that is relevant to the mass determination.

If you search through the atomic and nuclear tables very few examples are found. One possibility might be using



with a half life less than 500 years, but the most promising, studied in detail by De Rujula, seems to be



with a half life which has not been well determined. In this latter case the high-energy end of the photon spectrum will come entirely from L,M ... capture and have high intensity, so yielding excellent event rates.

CERN is particularly well suited to performing such an experiment.

First of all it possesses an isotope on-line separator (ISOLDE). The 600 MeV proton beam from the CERN synchrocyclotron is focused on a target ion source. A beam of radioactive ions is formed by a 60 kV acceleration stage and is mass analysed by a magnet. Individual mass isotopes can then be selected by electrostatic deflection, in particular the desired Holmium isotope crucial to the electron capture experiment.

The energy spectrum of the emitted photons can be studied with an accuracy of order 1 eV, and the shape of the high-energy tail thereby precisely determined.

It is known that (at least) three varieties of neutrino exist in Nature — ν_e , ν_μ , ν_τ of which the ν_e variety is produced in the above experiments. However, each of these is, in principle, a linear superposition of mass eigenstates ν_1 , ν_2 , ν_3 . As a result the ν_e is produced with various (unknown) probabilities to have mass m_1 , m_2 or m_3 . All three cases may affect the energy spectrum to produce a series of kinks. From the positions of the kinks it could be possible to determine the masses and relative probabilities for the various states.

The accumulation of isotopes is now in progress and the optimists claim that the experiment can be performed very quickly. It is possible that we have only a small time to wait before the neutrino masses are measured — unless some unforeseen experimental problems arise. □

Woodchuck, squirrel and duck hepatitis viruses

from Arie J. Zuckerman

THE close phylogenetic relationship between human hepatitis B virus and a virus isolated from the eastern woodchuck (*Marmota monax*) has been clearly established by Cummings and his associates (*Proc. natn. Acad. Sci. U.S.A.* **77**, 1842; 1980). They have cloned the DNA

Arie J. Zuckerman is Professor of Microbiology and Director of the WHO Collaborating Centre for Reference and Research on Viral Hepatitis at the London School of Hygiene and Tropical Medicine.

of human hepatitis B virus and the DNA of woodchuck hepatitis virus in the vector γ gtWES and subcloned into the kanamycin-resistant plasmid pAO1. Comparison of the recombinant DNAs with authentic virus DNAs by specific hybridization, size and restriction enzyme analysis indicated that the recombinants contained the complete genome of each virus. The nucleic acid homology between the two viral DNAs was confirmed with the cloned DNAs. Infection with either virus results in the accumulation in blood of

large amounts of excess virus coat protein in the form of spherical and tubular particles measuring 20–25 nm in diameter, and both viruses have 40–50 nm double-shelled or solid particles with a nucleocapsid or core containing double-stranded circular DNA with a gap and a virus DNA polymerase, and show a high degree of antigenic cross-reactivity between the cores of the two viruses, and a small region of 100–150 base pairs of nucleic acid homology as measured by liquid hybridization. The analogy between the two viruses is even closer when judged by their adaptation in their respective hosts causing persistent infection and close association with chronic liver disease and primary liver cancer.

Yet another virus related to human hepatitis B in Beechey ground squirrels (*Spermophilus beecheyi*) has been found in one region in northern California. Marion and her colleagues (*Proc. natn. Acad. Sci. U.S.A.* 177, 2941; 1980) identified a virus in these squirrels sharing many of the unique characteristics of the human virus. Common features include virus morpho-

logy, size and structure of the viral DNA, a virion DNA polymerase which repairs a single-stranded region in the double-stranded circular genome, cross-reacting surface viral antigens, antigen-antibody systems similar to hepatitis B e antigen and the core antigen, and persistent infection with viral antigen present continuously in the blood. Because the ground squirrels were only bled and then released, it has not been possible to observe a disease accompanying this virus in the ground squirrel.

There is also some preliminary evidence suggesting further members of this unusual class of viruses in black-tailed prairie dogs (*Cynomys ludovicianus*) and in ducks in a province in the People's Republic of China. Virological studies, however, have not yet been completed.

As the human hepatitis B virus has not been grown in tissue culture, these phylogenetically related animal viruses may provide valuable models for the study of viral replication and the pathogenesis of chronic liver disease and primary liver cancer. □

Hominoid habitats of the Miocene

from Peter Andrews

NEW STUDIES on the palaeoecology of fossil hominoids make it increasingly likely that fossil hominoids of the early Miocene, 18 to 20 million years ago, lived in quite different habitats from those of the middle Miocene, 12 to 15 million years ago, and suggest that the way of life of the ancestors of the great apes and man will have to be reconsidered. The early Miocene hominoid species are generally found associated with fossils indicating tropical forest conditions in contrast with the middle Miocene hominoids which are associated with temperate to tropical woodland. This ecological shift is accompanied by changes in tooth morphology — in particular the development of thick enamel — and in many other characters of the skull and postcrania. The early Miocene hominoids retain mainly primitive characters while the middle Miocene species have developed many more of the derived characters shared with the living great apes and man.

Hominoids in the middle Miocene were widely distributed in Europe and Asia along a broad belt stretching from southern Europe across to south-western China. Much of this region was covered with broad-leaved sclerophyllous woodland related to a vegetation type found today only in the Canary Islands (Axelrod *Ann. Miss. Bot. Gard.* 62, 280; 1975). The vegetation type was intermediate between

the tropical and subtropical forests to the south and the moist temperate woodlands with mixed conifers and broad-leaved trees to the north. It has not been clear to which part of this spectrum the middle Miocene hominoids were adapted, because direct associations between hominoid species and fossil floras have not, until recently, been available. Interpretations of past habitats by analysis of the animal faunas are limited, and at the *Ramapithecus* and *Sivapithecus* localities they do no more than indicate some type of woodland. However, a recently described flora from China that comes from the same beds as a mammalian fauna containing these hominoids provides the first direct evidence of the vegetation type associated with the middle Miocene thick enamelled apes.

The flora from Lufeng, in south-western China, shows a succession up the fossiliferous sequence from a warm humid vegetation type to cool temperate types (Sun Xiang-jun and Wu Yu-shu *Vert. Palae.* 18, 255; 1980). The middle part of the sequence, which is where the hominoids occur, is a lignite containing pollen of both aquatic and land plants. The lignitic nature of the deposits and the presence of aquatic plants suggest swamp conditions but by far the most abundant plant forms are temperate trees and shrubs represented by 44 taxa, with 8 taxa of ferns, 11 of herbs, and 3 aquatic forms. The trees and shrubs consist of both evergreen and deciduous taxa, and the composition of the

flora, although containing some sclerophylls, is closest to that of the temperate woodlands described by Axelrod as occurring to the north of the sclerophyllous belt during the middle Miocene. Woodlands of this type occur today in southern Europe to the north of the Mediterranean and Black seas. It is an unusual vegetation type in which to find primates, now largely tropical and subtropical in distribution and suggests that enamelled hominoids must have been able to survive in conditions beyond the range of most living primates.

Thick enamelled hominoids are known during the middle Miocene from Africa as well as from Europe and Asia. Faunas from Fort Ternan and Maboko Island, Kenya, have been analysed in another recent paper (Nesbit Evans *et al. J. Hum. Evol.* 10 (1), 1981), and demonstrate the presence of tropical woodland. The faunas have been analysed in two ways — by assessment of indicator species in the faunas, either singly or by combinations of species to produce habitat spectra, and by analysis of the community structure of the mammalian faunas through quantification of the diversity of ecological adaptations present in the faunas. The results of the two methods agree with each other, and with other evidence from the lower vertebrates and invertebrates, and assuming that the association between the hominoid species and the rest of the fauna is valid it can be inferred that the hominoids (*Ramapithecus* and/or *Sivapithecus*) also occupied woodland habitats during the middle Miocene in Africa.

The evidence for several early Miocene sites in Kenya is also reviewed in the same paper following the same methods. These confirm earlier results that at certain of the levels at Songhor, Rusinga Island and Koru, where the faunas are associated with hominoid species of the genera *Proconsul*, *Limnopithecus* and *Dendropithecus*, tropical forest habitat occurred.

The new data are of importance in considering the evolutionary emergence of the living great apes and man for they imply that their ancestors passed through an adaptive phase very different from their present way of life. *Sivapithecus* and *Ramapithecus* were the size of orang-utans and baboons respectively and animals of this size living in woodland habitats, whether tropical or temperate, must have been at least partly terrestrial because of discontinuities in the woodland canopy. Again, because woodland habitat shows greater seasonal variation than forest the supply of fruit would have been insufficient to have played more than a minor role in the diet. In conclusion, many previous studies that have claimed an arboreal and frugivorous ancestry for the living great apes and man will need to be reconsidered, for the evidence from Middle Miocene suggests that hominoids of this period were omnivorous and partially terrestrial. □

Variability of the Earth's rotation

from Frank D. Stacey

LONG BEFORE the advent of atomic clocks, it was well known that the Earth's rotation was an imperfect time-keeper. Precise time-keeping has always been one of the roles of astronomy and the stars, planets and satellites provide us with several independent or semi-independent clocks. Rather than clarifying the problem, modern measurements and recent re-analyses of earlier data have found evidence of a multiplicity of superimposed mechanisms. The difficulty is partly, of course, that some of the mechanisms involve time constants that are longer than the period of observation and so, regardless of the precision of observations, a long record is required to sort them out.

The diversity of disciplines that contribute to the study of the rotation of the Earth makes it an interesting theme for a wide-ranging discussion and, at a recent meeting*, astronomy, meteorology, oceanography, palaeontology, cosmology and the physics of the Earth's deep interior were all well represented. New data and ideas were forthcoming, as well as some thoughtful questioning of arguments supposed to be reasonably secure.

It now appears that at least some of the palaeontological data upon which we have relied for direct evidence of the relative lengths of the day, month and year in remote geological periods are very unsure. Certainly growth increments on the shells of marine organisms are well observed, but do they really indicate diurnal, monthly and annual periods, with all time increments faithfully recorded? Some of these questions have recently been brought to the fore by a controversial study of nautiloid growth rhythms (P. G. K. Kahn and S. M. Pompea *Nature* 275, 606; 1978; see also *Nature* 279, 452; 1979). W. W. Hughes (Andrew University, Michigan) has been examining a large number of nautiloid specimens from the British Museum (Natural History) collection. At least some organisms record the semi-diurnal lunar tide rather than the day and those observed under controlled conditions give no observable growth increments during the winter months. While we are not ready to give up hope of definitive data from growth rhythms, clearly we need much more work on control specimens and interpretation of ancient samples in the light of it before estimates of rotation rates in earlier epochs can be regarded as secure.

The gradual slowing of the Earth's rotation by tidal friction can be measured in several ways although the obvious and direct measurements of the apparent motion of distant stars with precise clocks (atomic or astronomical) is confused by shorter term fluctuations due to exchange of angular momentum within the Earth. It

is agreed that tidal friction in the Earth is dominated by the dissipation of tidal energy in the sea. To extrapolate to the remote past a linear relationship, in the sense that the lunar and solar tides act independently, has generally been assumed, with dissipation proportional to the square of tidal amplitude. For most purposes this may not be too bad an approximation, in spite of the evidence that tides are turbulent and therefore at least to some extent non-linear. However, when fine details of the angular momentum exchange with the lunar and solar orbits are sought, this assumption becomes unsatisfactory. For some years T. C. Van Flandern, at the Naval Observatory in Washington, has sought, with progressively increasing precision, evidence for a time variation of the newtonian gravitational constant, G , in terms of an atomic clock determination of the lunar orbital acceleration. It is important to know whether such an observation may be confused by non-linearity of the tide because the length of the record of atomic-timed observations of the Moon appears to be approaching the point of giving a definite result. According to V. Canuto (NASA Goddard Space Flight Center, *Nature* in the press) and G. M. Blake (University of Sheffield), the bias of presently available data favours an increase in G with time rather than the decrease postulated by P. A. M. Dirac.

Evidence of short-term variations in rotation is improving rapidly. A new analysis by L. V. Morrison (Royal Greenwich Observatory) and F. R. Stephenson (Liverpool University) of the timing of occultations, with some eclipse data back to 1620, gave a much more convincing length-of-day curve than any hitherto for the period from about 1750; the data before that time were of doubtful value. There are no discontinuities like those that appeared in earlier analyses, but the occurrence of changes amounting to a few parts in 10^8 over 5 or 10 years is strikingly confirmed. Irregular motions in the Earth's core are clearly implicated although the mechanism is a subject of controversy. A new examination from French observatory going back to 1700 of the correlation between rotational changes and the geomagnetic westward drift by J. L. Le Mouél and V. Courtillot (IPG, University of Paris) showed that magnetic changes preceded rotational changes by two or three years, contrary to some questionable analyses reported earlier. This indicates a semi-independent electromagnetic coupling of the Earth's solid mantle to the outer region of the core (which carries the westward drifting features of the field, as originally proposed by E. C. Bullard), and, more strongly, to the deeper body of the core. There are clearly difficulties with this hypothesis,

although it does provide a quantitative explanation of the rotation observations.

The time scale for core-induced changes in rotation rate is several years and a coupling time-constant of this magnitude is compatible with the electromagnetic torques exerted by the core-generated field on a lower mantle of conductivity $100 \Omega^{-1} \text{ m}^{-1}$ or so. However, the observations permit the supposition that core-mantle coupling is very tight indeed and that the apparent time constant is a feature of the core motions themselves. This supposition is necessary if the core has any role in exciting or damping the Chandler wobble. The conventional wisdom follows the Rochester-Smylie analysis of wobble coupling, which demonstrates that for the same field and mantle conductivity configuration the coupling time constant for the wobble is about 100 times as long as for the length-of-day changes. This is probably the strongest single reason for believing that tectonic displacements, including earthquakes, within the mantle excite the wobble, but S. K. Runcorn (University of Newcastle upon Tyne) keeps alive the argument that the core may be responsible for the wobble. The necessary mantle layer with a conductivity nearer to $10^4 \Omega^{-1} \text{ m}^{-1}$ is not well supported by observations, but such a layer does have proponents for other reasons, so this particular debate will continue for a while yet.

The smaller, but much more rapid, fluctuations in rotation must be attributed to atmospheric motions. A comprehensive new global analysis of the atmospheric angular momentum, reported by R. Hide, (Meteorological Office, Bracknell; see also *Nature* 286, 114; 1980; 286, 104; 1980) confirmed that major changes can occur quite rapidly, reacting on the Earth in a matter of days. The coupling is obscure but must certainly be there if the angular momentum exchange is demonstrated. A comparable analysis of the perpendicular components of atmospheric angular momentum, coupling to which would excite the wobble instead of changes in rotation rate, is promised. This reopens another of those debates that has been closed and reopened several times in the past 25 years.

What are the outstanding problems for the immediate future? If we compile a complete list, practically every problem that has ever been tackled is still on it. A personal list, restricted to four items in order of significance is (i) Adequacy of newtonian mechanics to explain the lunar orbit, (ii) Periodicities in the growth patterns of organisms exposed to a tidal cycle, (iii) Atmospheric excitation of the wobble, (iv) Lower mantle conductivity and core coupling. □

*Organized by Professor S.K. Runcorn for The Royal Astronomical Society on 9th January 1981.

Frank D. Stacey is Professor of Applied Physics at the University of Queensland, Australia.

REVIEW ARTICLE

Attenuation in the control of expression of bacterial operons

Charles Yanofsky

Department of Biological Sciences, Stanford University, Stanford, California 94305, USA

Bacterial operons concerned with the biosynthesis of amino acids are often controlled by a process of attenuation. The translation product of the initial segment of the transcript of each operon is a peptide rich in the amino acid that the particular operon controls. If the amino acid is in short supply translation is stalled at the relevant codons of the transcript long enough for the succeeding segment of the transcript to form secondary structures that allow the transcribing RNA polymerase molecule to proceed through a site that otherwise dictates termination of transcription. This site is the attenuator; the process is attenuation.

It has long been known that RNA polymerase, with and without accessory factors, recognizes specific nucleotide sequences which signal the initiation and termination of transcription, and that this recognition is exploited in various ways in the regulation of gene expression. But during the past 10 years it has become evident that transcription termination sites can be located within as well as at the end of an operon. These internal termination sites are used in the regulation of transcription and may be used to sense features and components of metabolism that cannot be used to control initiation of transcription. This mechanism of regulation, called attenuation, can be defined as the regulation of gene expression by selective reduction of the transcription of distal portions of an operon. Regulatory studies with bacteriophage λ (refs 1–5) revealed the first case of attenuation. Two operons of this phage contain transcription termination sites which are used to control transcription of the structural genes located immediately beyond. Soon after these studies with λ a different mechanism of attenuation was discovered in the tryptophan⁶ (*trp*) and histidine⁷ (*his*) operons of bacteria and subsequently in several other operons of the enzymes of amino acid biosynthesis. In this review I will discuss the mechanisms of attenuation, concentrating on the tryptophan operon of *Escherichia coli*.

During the early history of studies on gene expression in amino acid biosynthetic operons, thoughts on possible regulatory mechanisms were dominated by the conceptual contributions of Jacob and Monod. The focus of investigation was therefore on the detection of a repression system and on the relative roles of amino acid and charged transfer RNAs as activators of a presumed repressor protein^{8,9}. Nevertheless,

Ames and his co-workers concluded that transcription of the *his* operon of *Salmonella typhimurium* was probably not governed by a repressor protein and that tRNA^{His} rather than histidine was the signal molecule^{10,11}. Martin, Ames and Hartman¹², in some early speculations, proposed that it was translation of the transcript of the initial segment of the *his* operon that regulated transcription of the remainder of the operon. During the same period investigations on *trp* operon expression in *E. coli* followed a different course because there was convincing evidence that a tryptophan-activated repressor protein regulated transcription of the operon^{13–19}. However, several observations were inconsistent with the view that repression was the sole regulatory mechanism of this operon. Tryptophanyl-tRNA synthetase mutants were shown to have regulatory anomalies^{20–23}; addition of tryptophan to these strains did not fully depress *trp* operon expression. Moreover, Imamoto observed that the addition of tryptophan to tryptophan-starved cells not only shut down the initiation of transcription but also inhibited transcription in progress on the initial segment of the operon^{24,25}. Finally, mutants lacking a functional repressor could still respond to tryptophan starvation by increasing their rate of synthesis of *trp* messenger RNA^{26,27}.

These findings suggested that repression was not the sole means of regulating the *trp* operon of *E. coli*. Ultimately, two observations forced consideration of alternative regulatory schemes. Among a set of *trp* deletion mutants, in which both deletion termini were within the transcribed region of the operon, some had an unexpected sixfold increase in expression of the remaining genes of the operon^{6,28}. Since repressor control was not affected by these deletions, what must have been deleted was a site distinct from the operator but which was also used to regulate transcription of the operon. The second observation was that within the mRNA sequences produced *in vivo* from the 5' end of the *trp* operon, oligoribonucleotides corresponding to the first 140 base pairs of the operon were several times more abundant than those derived from more distal segments^{29,30}. These findings suggested that there was a site of transcription discontinuity, possibly a stop site, in the initial segment of the operon⁶.

When transcription of the initial segment of the *trp* operon was studied *in vivo* and *in vitro*^{28,30–34} it became apparent that a transcription termination site was located before the structural genes of the operon. Moreover, starving bacteria of tryptophan reduced termination of transcription at this site^{27,35}. Kasai provided convincing evidence of an equivalent site of regulation of transcription of the *his* operon of *S. typhimurium*⁷. He called regulation at this site 'attenuation', the term now generally used to describe regulation by transcription termination.

Definitions of terms

Leader region—The segment of an operon between the transcription start site and the structural gene(s).

Attenuator—a transcription termination site within an operon.

Transcription pause site—a region of DNA at which RNA polymerase pauses in the course of transcription.

Terminated leader transcript—The transcript terminated at the attenuator.

Termination structure—The segment of the leader transcript that is thought to be recognized as a termination signal by RNA polymerase.

Leader peptide—The short peptide encoded in the terminated and readthrough transcripts of the leader region.

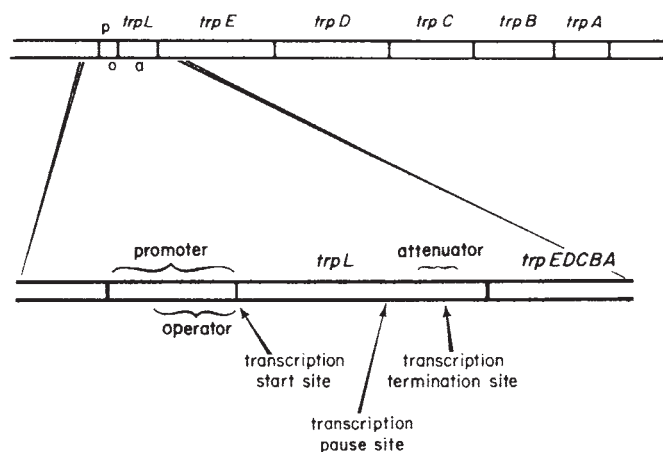


Fig. 1 The regulatory and structural gene regions of the *trp* operon of *E. coli*. Transcription initiation is controlled at a promoter-operator. Transcription termination is regulated at an attenuator, in the transcribed 162-base pair leader region, *trpL*. All RNA polymerase molecules transcribing the operon pause at the transcription pause site before proceeding further.

Features of the tryptophan operon and its regulatory region

The *trp* operon of *E. coli* consists of a transcription regulatory region and five structural genes encoding the polypeptides that catalyse the terminal sequence of reactions in tryptophan formation^{36,37} (Fig. 1). The operon is approximately 7,000 nucleotide pairs long, and each cistron of the polycistronic transcript of the operon seems to be translated equally. The initiation of transcription is regulated at an operator site located within the promoter region of the operon^{15-20,38,39}. A tryptophan-activated repressor protein, the product of the unlinked regulatory gene *trpR*, regulates initiation by controlling the access of RNA polymerase to the promoter³⁹⁻⁴¹. The transcription termination site or attenuator (see below for definitions of terms) is located within the 162-nucleotide pair transcribed leader region, *trpL*, preceding the first major structural gene. There is also a transcription pause site within *trpL* (M. Winkler and C. Yanofsky, in preparation). A possible role for this site will be discussed below.

Repression rather than attenuation exerts the greater quantitative effect *in vivo* on transcription of the structural genes of the operon; repression can reduce transcription up to 70-fold⁴² while attenuation can only reduce it 8-10-fold^{6,35}. Because tryptophan starvation increases expression by relieving both repression and attenuation, transcription of the *trp* operon of *E. coli* can be regulated over a range of about 600-fold. For a more thorough discussion of all aspects of regulation of tryptophan biosynthesis in bacteria the reader is referred to the excellent reviews of Crawford and Stauffer⁴³ and Platt³⁷.

Attenuation

In addition to locating the attenuator in the distal segment of *trpL*^{6,28}, mutants have also provided clues to the mechanism of attenuation. Mutations altering tryptophanyl-tRNA synthetase, tRNA^{Trp}, or a tRNA^{Trp} modifying enzyme and thereby reducing the efficiency of translation of Trp codons, were also found to decrease the termination of transcription at the *trp* attenuator⁴⁴⁻⁴⁷. This suggested that attenuation involved the translation of Trp codons rather than simply the recognition of changes in the tryptophan concentration. Attenuation in the *trp* operon is also affected by mutations of functionally important sequences in the leader region. Finally, mutations affecting RNA polymerase and the transcription termination protein rho also affect termination of transcription at the *trp* attenuator^{47,48}. The role of rho, if it has one, in transcription termination at the *trp* attenuator is unclear.

The leader transcript

Both *in vivo* and *in vitro* transcription studies established that transcription can be terminated in the leader region of the *trp* operon of *E. coli*^{28,32}. Termination occurs at approximately the same position *in vivo* and *in vitro* to give a leader transcript approximately 140 nucleotides in length³¹. The DNA region within which RNA synthesis stops is A + T rich and is immediately preceded by a G + C-rich region that exhibits dyad symmetry. This arrangement is typical of many prokaryotic transcription termination sites⁴⁹ and both regions are implicated in the transcription termination event. The question is how, at the *trp* operon attenuator, can tryptophan deficiency be sensed and communicated to the transcribing RNA polymerase molecule, allowing it to transcribe beyond the attenuator into the structural genes of the operon?

Ribosome binding experiments carried out with the 140 nucleotide *trp*-leader transcript yielded the unexpected result that ribosomes protect a 20-base segment of the early portion of the transcript from nuclease attack⁵⁰ (Fig. 2). A potential AUG start codon is located in the centre of the protected region. Were translation initiated at this start codon and terminated at the next stop codon (UGA at nucleotides 69-71), a 14-residue peptide would be synthesized with adjacent tryptophan residues near its distal end (Fig. 2). The presence of tandem tryptophan residues in this predicted 14-residue peptide was tantalizing because tryptophan is a rare amino acid in the proteins of *E. coli*, generally being used only once in every 100 amino acid residues. (The amino acid constitution of the predicted leader peptides now known to be specified by other biosynthetic operons (Fig. 3) turned out to be even more striking—see below.) The presence of the tandem tryptophan residues in this small peptide encoded in the leader region suggested a possible role for tRNA^{Trp} in the regulation of transcription termination at the *trp* operon attenuator³³. It was hypothesized that in cells starved of tryptophan a ribosome translating the leader transcript would stall at either of the two Trp codons. Only with sufficient tryptophan would the ribosome complete the entire leader peptide. The final position of the translating ribosome on the transcript could then be the distinctive feature communicated to the transcribing polymerase in the regulation of termination at the attenuator. It was reasonable to consider this hypothesis since in prokaryotes transcription and translation proceed in concert. These considerations raised two crucial questions. Is the potential AUG start codon within the ribosome-protected region (the ribosome binding site) actually used as a site of translation initiation, and, is the existence of adjacent Trp codons in the transcript coincidental?

The *trp* leader ribosome binding site was shown to be an efficient site for the initiation of translation by fusing it to a structural gene or a portion thereof and demonstrating the synthesis of fused polypeptides^{51,52}. Since the leader ribosome binding site is used, we must presume that our inability to detect

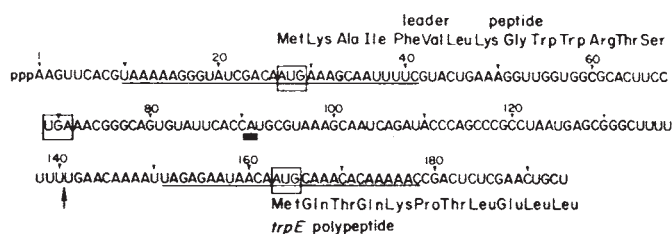


Fig. 2 The nucleotide sequence of the 5' end of *trp* messenger RNA. The non-terminated transcript is presented. When transcription is terminated at the attenuator, a 140-nucleotide transcript is produced. Its 3' terminus is marked by an arrow. The 3' terminus of the pause transcript, at nucleotide 90, is underlined by a bar. The two AUG-centred ribosome binding sites in this transcript segment are underlined. The boxed AUGs are where translation starts and the boxed UGA where it stops. The predicted amino acid sequence of the *trp* leader peptide is shown.

phe, *his*, *leu*, *thr* and *ilv* leader peptides

pheA: Met-Lys-His-Ile-Pro-PHE-PHE-PHE-Ala-PHE-PHE-PHE-Thr-PHE-Pro
his: Met-Thr-Arg-Val-Gln-Phe-Lys-HIS-HIS-HIS-HIS-HIS-HIS-HIS-Pro-Asp
leu: Met-Ser-His-Ile-Val-Arg-Phe-Thr-Gly-LEU-LEU-LEU-LEU-Asn-Ala-Phe-
 Ile-Val-Arg-Gly-Arg-Pro-Val-Gly-Gly-Ile-Gln-His
thr: Met-Lys-Arg-ILE-Ser-THR-THR-ILE-THR-THR-THR-ILE-THR-ILE-THR-THR-
 Gly-Asn-Gly-Ala-Gly
ilv: Met-Thr-Ala-LEU-LEU-Arg-VAL-ILE-Ser-LEU-VAL-VAL-ILE-Ser-VAL-VAL-
 VAL-ILE-ILE-ILE-Pro-Pro-Cys-Gly-Ala-Ala-Leu-Gly-Arg-Gly-Lys-Ala

Fig. 3 The predicted amino acid sequences of the leader peptides of the *pheA*, *his*, *leu*, *thr* and *ilv* operons of *E. coli* or *S. typhimurium*. Amino acids which regulate the respective operons are in italicized capitals and are underlined. For references see text.

the leader peptide is due to its instability. That the leader peptide as such does not have a regulatory role is indicated by complementation analyses⁵³.

The significance of the tandem Trp codons in the *trp* leader transcript of *E. coli* is supported by their presence also in the *trp* leader regions of *Shigella dysenteriae*, *Salmonella typhimurium*, *Serratia marcescens*^{34,54,55} and *Klebsiella aerogenes* (M. Blumenberg and C. Yanofsky, unpublished). The predicted amino acid residues of these *trp* leader peptides are also conserved in the Arg-Thr-Ser sequence that follows the adjacent Trp residues but not in the sequence that precedes it. More importantly, the predicted leader peptides of other amino acid biosynthetic operons that are regulated by attenuation also are rich in the amino acid which is the end product of the appropriate pathway (Fig. 3). Hence, in a bacterial cell starved of any of these amino acids, the ribosome translating the leader transcript of the respective operon would stall over the appropriate codon rather than move to the leader transcript stop codon. Figure 3 reveals that the number as well as the location of the distinctive amino acids varies, presumably reflecting quantitative differences in regulation of the different operons.

If ribosome stalling at the Trp codons of the transcript of the leader region were sufficient to prevent the termination of transcription at the *trp* attenuator, then would stalling anywhere in the coding region of the transcript have the same effect? Starving *E. coli* mutants for each of seven amino acids encoded in the *trp* leader transcript (Met, Val, Leu, Gly, Trp, Arg and Thr) and two that are not (His and Pro)⁵³ revealed that the starvation response was relatively specific. Only cells depleted of Trp or Arg exhibited reduced termination of transcription at the *trp* attenuator. Comparable starvation experiments with the *trp* operon of *S. marcescens* (I. Stroynowski and C. Yanofsky, unpublished) demonstrated that starvation for His, the second residue before Trp in the leader peptide, as well as for Trp or Arg, reduced the termination of transcription at the *trp* attenuator. These observations suggested that ribosome stalling in the vicinity of the Trp codons is sufficient to exert a regulatory effect.

This conclusion led to the question of how amino acid starvation and presumed ribosome stalling are communicated to the transcribing polymerase. Hoping to answer this question we scrutinized the transcript of the leader region, searching for potential secondary structures that might relate to the transcription termination event. This search was prompted by the suggestion of Marty Rosenberg, on the basis of sequence analyses with a terminated transcript of bacteriophage λ ⁵⁶, that base-paired segments in RNA may serve as a signal in the transcription termination event. Limited RNase T₁ digestion of the 140-nucleotide long *trp* leader transcript revealed extensive secondary structure in the transcript; many phosphodiester bonds in the latter half of the transcript were relatively resistant to nuclease attack³³. Also, certain fragments in partial digests of the transcript were observed to migrate together during electrophoresis in non-denaturing gels⁵⁷. Presumably they did so

because they were base-paired. Analyses of the nuclease-resistant RNA fragments, as well as computer matching of complementary sequences, indicated that the transcript contains the two hairpin loops shown in Fig. 4 (left). For simplicity, we designate the four strands participating in these structures 1–4 (Fig. 4). We observed that strands 1 and 2 were paired and that strand 3 remained covalently attached to strand 4. The calculated free energies of these base-paired structures are given in Fig. 4. Note that strand 1 contains the adjacent Trp codons and that structure 3:4 resembles other hydrogen-bonded hairpin structures that immediately precede the 3' ends of terminated transcripts. Inspection of the sequences of the paired regions revealed an interesting feature—that strand 2 could potentially pair with strand 3 as well as with strand 1³³ (Fig. 4, right). This led to the suggestion that alternative secondary structures in the *trp* leader transcript might provide the termination or read-through signals that RNA polymerase recognizes.

We could imagine, as shown in Fig. 5, that the position of the ribosome on the transcript determines which one of the alternative RNA secondary structures would be formed^{46,57}. For example, a ribosome stalled at the Trp codons or the Arg codon by a shortage of either amino acid would sterically block 1:2 pairing, thereby allowing strand 2 to pair with strand 3 as soon as strand 3 had been synthesized. By the time strand 4 had been synthesized its potential pairing partner, strand 3, would be base-paired and unavailable, preventing the formation of termination structure, 3:4. If 3:4 did not form and if, as we also believe (see next section), the 3:4 structure is the termination signal that RNA polymerase recognizes, then the transcribing polymerase would not stop at the attenuator. Consequently, the structural genes of the operon would be transcribed, leading ultimately to the biosynthetic correction of the tryptophan deficiency. If, however, a ribosome stalled at the Thr codon or reached the stop codon, strand 2 would be sterically prevented from pairing with strand 3 and thus 3:4 would be free to form and signal transcription termination. If the ribosome stalled at the Gly codon strand 1 would pair with strand 2, thereby preventing 2:3 pairing and facilitating pairing of 3 with 4. According to this scheme it is the location of the ribosome on the transcript of the *trp* leader region that determines which alternative RNA secondary structure forms. This, in turn, directs RNA polymerase to terminate transcription or to read through

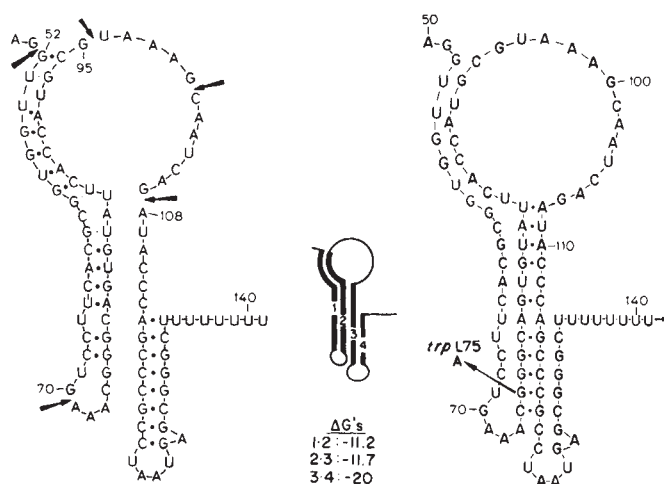


Fig. 4 Secondary structure alternatives in the *trp* leader transcript. In the large structure on the left the two base-paired structures that are detected *in vitro* are presented. The arrows indicate the sites of RNase T₁ attack. The G-bonds in the hydrogen-bonded regions are not cleaved, presumably because the Gs are base paired. In the large structure on the right an alternative secondary structure is shown. Formation of this structure is thought to prevent transcription termination at the attenuator. For simplicity we designate the transcript segments that participate in these hydrogen-bonded structures strands 1–4 (see centre inset). The *trp* codons are in strand 1. The *trpL75* mutation is indicated.

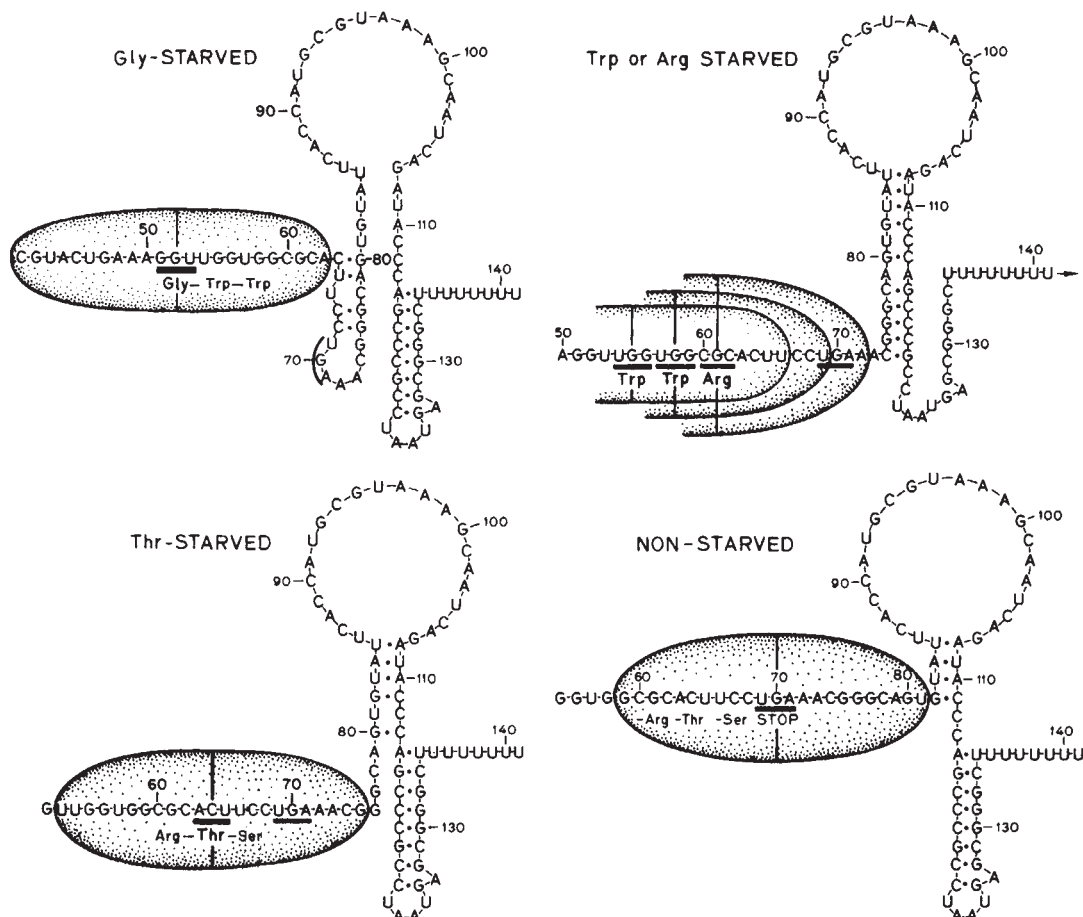


Fig. 5 Schematic representation of ribosome position on the *trp* transcript in cells starved of Gly, Trp, Arg or Thr, or cells that are not starved. It is proposed that a ribosome stalled over one of the codons of the transcript sterically blocks about 10 nucleotides on the downstream segment of the transcript, thereby determining which alternative secondary structure will form during transcription. Only when cells are starved of Trp or Arg is the ribosome positioned so that structure 2:3 will form. The existence of structure 2:3 could prevent pairing of 3 with 4 while 4 is being synthesized. It is assumed that transcription terminates at the attenuator only when structure 3:4 is formed during transcription.

into the structural genes of the operon. With this model for reference we can explain why the Arg-Thr-Ser codon sequence that follows the tandem Trp codons of *trp* transcripts is conserved in the enterobacteria. The Arg-Thr-Ser coding region is within the segment of strand 1 that can base pair with strand 2 (Figs 4, 5). Conservation of this region presumably reflects a requirement for this pairing capability.

Mutations in the leader region of the *trp* operon

Two classes of termination-defective *trp* leader mutants have been isolated. One type terminates transcription at less than the normal frequency and therefore operon expression is increased^{58,59}. The base-pair changes in approximately 30 mutants of this type have been determined and, with one exception, all are in the segment of the leader region that corresponds to the 3:4 base-paired RNA structure (Fig. 6). Each of these base-pair changes reduces the predicted stability of the 3:4 structure by about 50%. All of these mutations affect the central G-C base pairs of the structure (Fig. 6), which of course have the greatest effect on stability of the 3:4 structure. *In vivo*, each of these mutations results in a two- to fourfold increase in operon expression. When restriction fragments bearing these mutations are transcribed, 40–70% of RNA polymerase molecules read through the attenuator. With a wild-type template only 5% do so. Clearly, these single mutant changes markedly influence the termination of polymerase activity. Largely on the basis of these findings we believe that the hydrogen-bonded 3:4 structure is the termination signal that the transcribing polymerase recognizes. Consistent with this conclusion is the additional observation³³ that when transcription is carried out *in vitro* with ITP substituting for GTP, transcription is no longer terminated at the attenuator, presumably because I=C base-paired RNA is less stable than G=C base-paired RNA. Similar *in vitro* transcription studies

with other base analogues that affect RNA secondary structure also suggest that a stable 3:4 RNA secondary structure is necessary for transcription termination⁶⁰.

A role for the A+T-rich as well as the G+C region of the attenuator in termination of transcription is suggested by our discovery of a termination-defective *trp* leader mutant with a single base-pair change in the A+T-rich region of the attenuator⁵⁸. In addition, a deletion mutant exists in which the distal four A+Ts of the A+T-rich region are replaced by a foreign sequence³¹. This change eliminates transcription termination at the attenuator *in vitro* and reduces it *in vivo*^{31,60}. Certain mutants altered in the β subunit of RNA polymerase are also aberrant in termination of transcription at the *trp* attenuator (C. Yanofsky and V. Horn, unpublished). In one class termination is partially relieved, while in a second, termination is more frequent. These mutations influence the termination of transcription only in strains which can form the 3:4 base-paired structure, strengthening the conclusion that this structure is the termination signal that is recognized by RNA polymerase.

There are also mutations in the *trp* leader region which increase termination at the attenuator *in vivo*⁵³. Some mutations of this type have a second interesting and important property; they prevent the relief from termination that is normally associated with tryptophan or arginine starvation⁵³. This observation provides the strongest evidence for our model of attenuation. In one of these mutants, *trpL29*, which has been isolated on two separate occasions, the AUG start codon for the leader peptide is replaced by AUA, a change which would prevent efficient translation of the leader transcript. Since strains with this mutation do not respond to tryptophan starvation by relieving termination, a prediction of our model—that a ribosome must move to the Trp codon region of the transcript for transcription termination to be relieved—is supported. A second type of mutant, *trpL75* (Fig. 4), of which there have also been two isolates, has a G→A change at position 75 in the leader region. Since this change essentially eliminates the pairing of strand 2 with strand 3, thereby leading to the formation of the 3:4

termination structure, our model explains why termination occurs despite tryptophan starvation. These considerations support two important features of our model: first, that a ribosome must translate the transcript at least to the first Trp codon for termination relief, and second, that pairing of strand 2 with strand 3 is also required for this relief. Thus, these observations strongly suggest that alternative secondary structures in the RNA transcript determine whether or not termination will occur at the *trp* operon attenuator.

Why do these two types of mutants exhibit increased termination of transcription at the attenuator *in vivo*? In the wild-type bacterium we assume that operon expression provides for some transcription readthrough, even in the presence of excess tryptophan⁵⁸. This could be due to rapid dissociation of the translating ribosome from the stop codon of the transcript, allowing the 2:3 alternative structure to form. If this happens, formation of the 3:4 structure would be prevented and the transcribing RNA polymerase molecule would continue into the structural genes of the operon. This model can explain the increased termination observed *in vivo* in the two types of termination mutants. The AUA translation initiation-defective mutant would, during transcription, form structure 1:2. This would prevent pairing of strand 2 with 3, thereby promoting the formation of termination structure 3:4. In mutant *trpL75*, pairing of strand 2 with strand 3 is presumably defective. Hence, despite ribosome dissociation from the stop codon, strand 2 could not pair with strand 3, and therefore 3:4 would form more often. Thus, the increased termination of transcription observed in these mutants can be readily accommodated within our model.

Also consistent with our model is the observation that neither mutant change affects transcription termination at the attenuator *in vitro*⁵⁸.

The leader pause site

When restriction fragments containing the *trp* promoter are transcribed we observe the 140-nucleotide terminated leader transcript as the major product along with a small amount of a readthrough transcript. However, at early times during a single round of transcription, a third, major transcript is seen (M. Winkler and C. Yanofsky, unpublished). This species—which we call the pause transcript—is 90 nucleotides long and has at its 3' terminus the sequence that could form the 1:2 base-paired structure (Fig. 4). Enhancement of pausing at this site when BrUTP replaces UTP (P. J. Farnham and T. Platt, unpublished) supports this proposal since numerous U's are present in the 1:2 stem. Kinetic studies indicate that every polymerase molecule that transcribes the initial segment of the leader region pauses at the 'leader pause site'. Eventually, these polymerase molecules resume transcription so that the pause transcript grows to become either the terminated transcript or

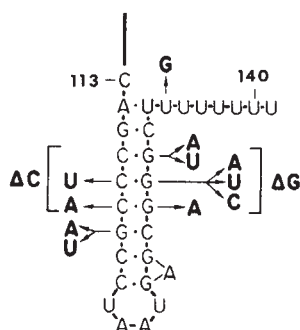


Fig. 6 Base pair changes in the *trp* leader region that relieve transcription termination at the attenuator. The changes in the transcript are shown; all are in the 3:4 structure and reduce its stability except the single U → G change at position 135.

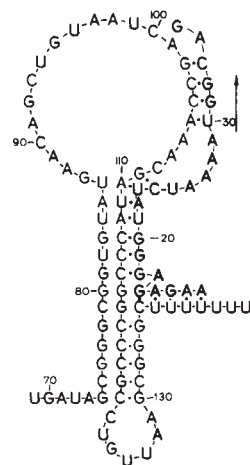


Fig. 7 Pairing of the leader ribosome binding site with the distal portion of the *trp* leader transcript of *S. typhimurium*. Note that both the Shine-Dalgarno region and the start codon region are hydrogen bonded to the distal segment of the transcript. Pairing of the indicated RNA segments has been demonstrated experimentally (K. Brown and C. Yanofsky, unpublished).

the readthrough transcript. The position of the pause site in the leader region suggests that it could be used to synchronize ribosome attachment, movement and positioning on the leader transcript relative to the transcribing RNA polymerase molecule. The polymerase molecule might be held at the pause site until the translating ribosome reached a particular position on the transcript. Thereafter, the polymerase molecule would resume transcription and the translating ribosome would reach the positions on the transcript that favour one or the other of the alternative RNA secondary structures.

The leader ribosome binding site

Perusal of the sequences of the *trp* leader transcripts of various organisms and the results of secondary structure studies with these transcripts suggest an additional feature of the attenuation process. When the secondary structures formed in *trp* leader transcripts from different enterobacteria were analysed, another base-paired species was detected in addition to the 1:2 and 3:4 structures (K. Brown and C. Yanofsky, in preparation). This species contains an early segment of the transcript—the region containing the leader ribosome binding site—base-paired to a distal segment (Fig. 7). The calculated free energy for this base-paired segment is about $-32 \text{ kcal mol}^{-1}$. If this pairing occurs *in vivo* the distal segment of the transcript could block the leader ribosome binding site, thereby preventing additional rounds of translation. Thus, once the decision was made to terminate transcription or to allow readthrough at the *trp* attenuator, additional translation of the peptide coding region would be prevented by masking the leader ribosome binding site. Sequence arrangements that allow pairing with the leader ribosome binding site may be a common feature of the structure of transcripts of operons regulated by attenuation (ref. 54 and M. Blumenberg and C. Yanofsky, unpublished). This situation is reminiscent of the masking of ribosome binding sites in RNA phages^{61,62}.

Summary of the mechanism of attenuation

Our current view of the events accompanying attenuation in the *trp* operon is as follows: a RNA polymerase molecule that escapes repressor control initiates transcription of the operon.

Soon after the segment of the transcript containing the leader ribosome binding site has been synthesized a ribosome attaches. Synthesis of the leader peptide begins while transcription of the later regions continues. The transcribing RNA polymerase molecule reaches the transcription pause site where it remains briefly, allowing the translating ribosome to approach. When RNA polymerase resumes transcription, the translating ribosome either stalls at one of the Trp codons, if the cell is deficient in tryptophan, or, if tryptophan is plentiful, moves to the translation stop codon. The position of the ribosome on the transcript determines which alternative RNA secondary structure forms. Only if structure 3:4 forms does the polymerase terminate transcription at the attenuator. Whether termination occurs or not, the leader ribosome binding site is then blocked by pairing with a transcript segment.

The interpretation given above presumes that changes in the secondary structure of transcripts mediate the regulatory response. This has not been proved! Structural studies on transfer RNAs have established that interactions other than hydrogen bonding contribute to three-dimensional form. Furthermore, a regulatory role for the DNA template has not been excluded. We should therefore keep open the possibility that other interactions also participate in establishing the informational transcript or template structures that are crucial in attenuation.

Attenuation in other amino acid biosynthetic operons

Extensive regulatory studies have been performed with the *his* operons of *S. typhimurium* and *E. coli*. This operon was the first gene cluster for which there was convincing evidence suggesting that charging of a transfer RNA had a regulatory role⁸. The leader regions of the *his* operons of *S. typhimurium* and *E. coli* have been sequenced^{63,64} and appear to have structures resembling that of the *trp* operon. A potential ribosome binding site is situated in the leader transcript sequence, which, if used, would give a 16-residue peptide containing 7 adjacent His residues. A G+C-rich region with dyad symmetry is located somewhat beyond and is followed by an A+T-rich segment within which transcription termination probably occurs. The transcript segment corresponding to the G+C-rich region could form a stable stem and loop that could serve as a termination signal. A potential alternative competing secondary structure can be drawn for the transcript that could prevent formation of the presumed termination structure, depending on the location of the ribosome translating the leader peptide-coding region⁶³⁻⁶⁵. *In vitro* studies have implicated translation in the regulation of transcription of the *his* operon⁶⁶. In addition, analyses with regulatory mutants altered at key positions in the *his* leader region provide insight into the parts played by different segments of this region or more likely, the corresponding transcript⁶⁵. An ochre nonsense mutation in the leader peptide-coding region decreases operon expression to the extent that the corresponding mutant is a His auxotroph. This observation suggests that translation of at least the initial part of the leader peptide coding region is essential for operon expression. Suppression of the ochre mutation increases *his* enzyme levels and restores the His⁺ phenotype. A second mutation conferring His auxotrophy occurs just beyond the translation stop codon for the leader peptide. Oddly enough, although this mutation does not introduce an amber codon, it is suppressed by amber suppressors. Apparently, the mutation prevents formation of the competing RNA structure, resulting in more efficient termination at the attenuator⁶⁵. Presumably, the amber suppressors increase operon expression by allowing the translating ribosome to translate the leader peptide stop codon and disrupt the transcript termination structure⁶⁵. One verified prediction of this interpretation was that amber suppressors would increase expression of the wild-type *his* operon.

Finally, a deletion that removes part of the termination structure increases operon expression. Thus, all aspects of attenuation in the *his* operon are similar to those of the *trp* operon, suggesting that attenuation is used in much the same way.

The *thrA₁A₂BC* and *ilvGEDA* operons of *E. coli* have an additional feature readily accommodated by the attenuation mechanism regulating amino acid biosynthetic operons⁶⁷⁻⁶⁹. Expression of these two operons is influenced by two and three amino acids, respectively (multivalent control). Obviously, if a leader transcript contains multiple codons for two or three amino acids in the critical pairing regions, the operon could respond to starvation for any one of these amino acids. The sequences of the leader regions of the *thr* and *ilv* operons suggest that this is exactly what occurs. The *thr* leader region encodes a peptide rich in Thr and Ile residues (Fig. 3); it contains a G+C-rich, A+T-rich region resembling other terminators, and its sequence predicts the formation of alternative, competing paired transcript structures that would be influenced by ribosome position on the transcript⁶⁷. Mutants establish that the G+C-rich region is essential for the termination of transcription. The *ilvGEDA* operon is a more extreme example of multivalent control. It is regulated by changes in the extent of charging of Val, Ile and Leu tRNAs⁸. The leader region codes for a putative 32-residue peptide with multiple Ile, Val and Leu residues and contains adjacent G+C- and A+T-rich regions at which transcription is terminated *in vitro*^{68,69}. Mutually exclusive secondary structures have been predicted for the transcript. Their role in the termination of transcription is supported by the finding that substitution of ITP for GTP in the *in vitro* transcription reaction prevents termination. The potential secondary structures in the transcript are complex, perhaps reflecting the need to respond to starvation for any one of the three amino acids. Stalling of more than one ribosome on the transcript may be required for maximum relief of termination⁶⁸.

The *leu* operon of *S. typhimurium* has a leader region containing all the features found in the other amino acid operons^{70,71}. A 160-nucleotide terminated transcript is produced *in vitro*. The transcript codes for a 28-residue peptide containing 4 contiguous Leu residues. Alternative secondary structures can be predicted for the transcript, including a termination structure, a competing structure and a third structure that could prevent formation of the competing structure if translation of the transcript stopped early in the peptide-coding region. Again, substituting ITP for GTP in the *in vitro* transcription reaction prevents termination.

The Leu codons of the transcript are so positioned that ribosome stalling in the Leu codon region would promote formation of the RNA structure that is the alternative to the termination structure.

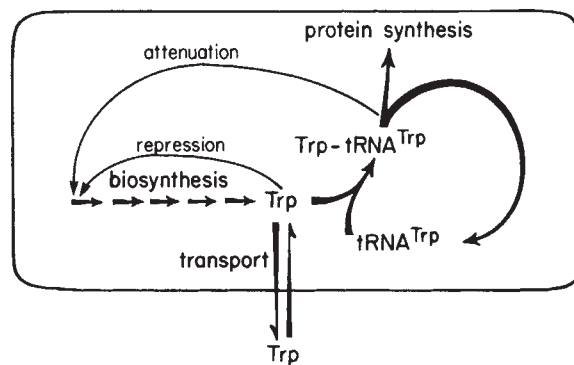


Fig. 8 Metabolic processes involved in tryptophan synthesis and utilization. The existence of the two transcription control mechanisms for the *trp* operon allows the bacterium to sense both the intracellular concentration of tryptophan and the extent of charging of tRNA^{Trp}.

The *pheA* operon of *E. coli* is a single gene operon containing a leader region with all the features seen in other amino acid operons controlled by attenuation⁷². A terminated transcript is produced *in vitro* and the site of termination is a typical termination region. The predicted *phe* leader peptide is 15 residues in length and contains 7 Phe residues in the last two-thirds of the peptide. Stable RNA secondary structures can be drawn for the *phe* transcript. Ribosome stalling over the Phe codons of the transcript could prevent formation of the transcript termination structure.

Thus, despite considerable differences in the sequences of leader regions of the *trp*, *his*, *thr*, *ilv*, *leu* and *phe* operons, the predicted structures of each of their transcripts allows the same mechanism of attenuation. When we compare the specific elements of repression systems with those of attenuation as they are used in amino acid biosynthetic operons, it is obvious that from the point of view of information content attenuation is a more economical regulatory mechanism. The unique regulatory information of attenuation is provided by the sequence of 50 or so base pairs of the leader region of each operon. This information is sufficient to be interpreted in terms of the appropriate regulatory response by the transcriptional and translational machinery of bacterial cells.

Conclusions

The existence of two mechanisms for regulating transcription of the *trp* operon leads one to wonder what advantage it is to the organism to have this apparent duplication of regulatory effort. The two mechanisms clearly allow similar responses to the same physiological situations. A possible explanation can be offered by considering the various metabolic reactions involved in the biosynthesis and utilization of tryptophan (Fig. 8). The repression system, by responding to tryptophan exclusively, is tailored to titrate the intracellular concentration of this amino acid. This concentration depends on several factors: first, tryptophan availability in the environment of the bacterium and its entry into the cell via transport systems; second, the rate of tryptophan biosynthesis; and third, the utilization of tryptophan for protein synthesis. By gauging the intracellular concentration of tryptophan, repression is particularly well suited to regulate expression of the operon in response to changes in the availability of tryptophan from the environment. We believe this to be its primary purpose. Attenuation is influenced by the extent of charging of tRNA^{Trp}. The relative concentrations of charged and uncharged tRNA^{Trp} are determined by several factors: the intracellular tryptophan concentration, the amount and activity of tryptophanyl-tRNA synthetase, the amount of tRNA^{Trp} and, perhaps most importantly, the overall rate of protein synthesis. It is this last factor that we believe provides the best explanation for attenuation. If a cell becomes deficient in one of the other amino acids the tRNA^{Trp} pool will become fully charged, resulting in maximal termination at the attenuator. Conversely, if a cell suddenly begins to synthesize proteins rapidly and tryptophan is in short supply, the tRNA^{Trp} pool will be relatively uncharged and there will be little or no termination at the attenuator. Thus, the attenuation mechanism nicely regulates expression of the operon relative to the overall rate of protein synthesis. The combined action of the two regulatory mechanisms enables the bacterium to recognize and respond to the principal external and internal events relevant to expression of the operon.

If it is advantageous to have two transcription control mechanisms for the *trp* operon of *E. coli*, why is attenuation the sole transcription control mechanism used to regulate expression of other amino acid biosynthetic operons such as the *his*, *leu* and *thr* operons^{65,67,70}? The explanation for this apparent contradiction, I believe, is provided by our recent finding that two other operons are regulated by the *trp* repressor. These are the *aroH* operon, specifying one of three enzymes that catalyse the initial reaction in the common pathway of aromatic amino acid biosynthesis⁷³, and the *trp* repressor operon, encoding the

trp aporepressor^{40,74}. In both cases, tryptophan-activated repressor binds to the operator-promoter region of the operon and inhibits transcription initiation. However, the operators in these three operons are at different locations in their respective promoters⁴⁰. This finding is consistent with the hypothesis that these operators evolved independently. Thus, we might speculate that in an organism ancestral to *E. coli* and other enterobacteria, the *trp* repressor might have been used to regulate *aroH* expression while the *trp* operon was regulated by attenuation alone. Subsequently, by very few mutations, an operator may have evolved in the *trp* operon, thereby permitting additional control over transcription of this operon. We suggest, therefore, that two regulatory mechanisms now exist for controlling transcription of the *trp* operon of *E. coli* because it was advantageous to the organism to have evolved a *trp* repressor binding site in this operon so that it could exploit the availability of the *trp* repressor.

Extensions

Attenuation as used in the regulation of amino acid biosynthetic operons is only one of several ways in which gene expression could be controlled through the termination of transcription. Alteration or modification of RNA polymerase, as is presumed to occur when it interacts with the N protein of bacteriophage λ ⁷⁵⁻⁷⁸, or interference with the action of proteins participating in transcription termination⁷⁸, are additional attenuation mechanisms. Transcription termination sites could also be used simply to reduce transcription of a gene relative to a leader segment or a preceding gene in the same operon. The *trpR* operon, specifying the *trp* aporepressor, has a termination site in its leader region which may function in this manner⁴⁰. Similarly, the termination site located between *rplL* and *rpoB* in the *rplJL-rpoBC* operon may be used to regulate expression of the two RNA polymerase structural genes relative to the ribosomal protein genes⁷⁹. Inadvertent attenuation also occurs when translation is prematurely terminated within one of the structural genes of a polycistronic operon. Here, in what is termed translational polarity⁸⁰, the termination of transcription reduces the transcription of distal genes⁸¹. In addition to these examples, one could imagine many interactions that would alter the secondary structure of RNA and thereby influence termination. Transcriptional pausing⁸²⁻⁸⁴ should also be considered a mechanism of attenuation. Pause sites could conceivably be used to set the rate of transcription of a gene or operon below that attainable by polymerase-promoter interaction alone. Pause sites may thus fix the maximum rates of transcription. Studies on pausing in phage T7 and in the *trp* operon suggest that pausing can serve different regulatory roles^{83,84} (M. Winkler and C. Yanofsky, unpublished). Finally, attenuation may not be limited to bacteria and their viruses but may also occur in animal viruses. In vesicular stomatitis virus (VSV), transcription decreases in an oriented manner at or near the junctions of neighbouring genes, resulting in a cumulative reduction in distal gene expression⁸⁵. In adenovirus and VSV transcription short leader transcripts are accumulated^{86,87}, an observation suggesting attenuation. It is apparent from the examples cited that transcriptional attenuation is accomplished in diverse ways. We presume that these mechanisms bring into regulatory focus those cellular processes and components that cannot readily participate in the mechanisms that control the initiation of transcription.

Perusal of the reference list will convince the reader that the model developed in this article was based on the work of a large number of collaborators. To each, I express my sincerest appreciation for sharing in this adventure. I also thank my current co-workers and Irving Crawford, Terry Platt, Howard Zalkin and George Stauffer for their helpful comments, and the NSF, the USPHS and the American Heart Association for their continuing support. C.Y. is a Career Investigator of the American Heart Association.

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ARTICLES

VLBI structures of the images of the double QSO 0957+561

R. W. Porcas

Max-Planck-Institut für Radioastronomie, Auf dem Hugel 69, Bonn, FRG

R. S. Booth, I. W. A. Browne, D. Walsh & P. N. Wilkinson

University of Manchester, Nuffield Radio Astronomy Laboratories, Jodrell Bank, Macclesfield, Cheshire SK11 9DL, UK

Very long baseline interferometry (VLBI) observations of the double QSO 0957+561 have revealed radio fine structure in the two 'image' components A and B. The structures are similar, both of the 'core-jet' type typical of many compact extragalactic radio sources. The shapes of the images put strong constraints on the mass distribution responsible for the gravitational imaging.

THE twin QSOs 0957+561A, B are widely believed to be images of a single QSO produced by the gravitational lens effect¹. The lens has been identified with an 18.5 mag elliptical galaxy in a distant cluster^{2–5}. The similarity of the optical spectra of the images has been confirmed^{6,7} and the flux ratio of the

images has been shown to be the same over a wide range of frequencies covering the radio to UV parts of the spectrum^{8–17}.

The asymmetry of the geometry of the 'images' A and B with respect to the galaxy indicates that the lens action cannot be modelled by a spherical mass distribution³. The gravitational

field of the surrounding galaxy cluster and the (non-spherical) mass distribution of the elliptical galaxy also determine the geometry of the images. In previous VLBI observations¹⁰ we showed that the radio components A and B both have compact structure on a scale size of <20 marc.s. We now report the results of more extensive and more sensitive VLBI observations at two frequencies, using interferometers with resolutions ranging from 60 to 1 marc.s.

Observations

We have made two sets of VLBI observations of the double QSO with the 2-MHz bandwidth Mark II recording system, using a transatlantic baseline and the large antennas available in the European VLBI network. On 9–10 January 1980 we observed at 4,996 MHz using the Max-Planck-Institut für Radioastronomie (MPIfR) 100-m telescope at Effelsberg and the NRAO 43-m telescope at Green Bank, West Virginia. On 22–23 February we observed at 1,666 MHz using the Effelsberg and Green Bank antennae, and also the Jodrell Bank 76-m and Onsala 25-m antennae. Hydrogen maser frequency standards were used at Effelsberg, Onsala and Green Bank, and a rubidium standard at Jodrell Bank. All the cross-correlation of the signals for these experiments was done with the MPIfR processor in Bonn. In the subsequent analysis we attempted to achieve the highest possible sensitivity by integrating the cross-correlation function for as long as the coherence of the local oscillators, atmosphere and ionosphere would allow. This coherence time was determined by observation of the calibration sources 1642+69, BL Lac and 0235+16, which also served to calibrate the gain of the interferometers.

With the resolution used in these observations the double QSO consists only of two compact 'image' components A and B and there is no evidence of any compact structure associated with the extended components seen in lower-resolution maps. The A and B components are characterized by separate residual fringe rates in the cross-correlation function, representing their offsets from a reference 'fringe stopping' position. The long integration times then permit a clear separation of the visibility functions of A and B in the fringe rate spectrum for all but a few periods of time on each baseline (see ref. 10). Data corresponding to these periods were not used in the analysis.

Image structures

The highest sensitivity was obtained at 1,666 MHz. With 10-min integration times, the r.m.s. noise errors on the baselines Effelsberg–Jodrell, Onsala–Effelsberg and Onsala–Jodrell were 3, 6 and 6 mJy, respectively, with minimum lobe spacings in the range 30–60 marc.s. On the Effelsberg–Green Bank baseline, which had resolution 6 marc.s, the noise level was 5 mJy after 20 min integration. The visibility functions obtained for A and B on these baselines are shown in Fig. 1a–h.

The 1,666-MHz data show that A and B exhibit remarkably similar behaviour, both visibility functions indicating structure on the scale of ~ 50 marc.s. The data for the baseline Effelsberg–Jodrell can be compared directly with that reported in Porcas *et al.*¹⁰ which was taken at times corresponding to UT 16–19 h and 0.5 h in our present observations. These are all times which, by chance, were well away from the minima in the visibility functions and we were unable then to attribute the small amplitude differences to source structure.

To derive the source structures from the visibility functions we have fitted simple two-component gaussian models to the 1,666-MHz data. The data from both A and B are fitted well by models consisting of a barely resolved (core) component and an elongated (jet) component. The parameters of the best fitting models are given in Table 1. The visibilities predicted by the model are shown by the solid lines drawn through the data points in Fig. 1. Table 1 also shows errors where the parameter values are well defined by the data. This applies in particular to the position angle (PA) and separation of the jets from the cores in both A and B. Other parameters are less well defined because of strong coupling between their values, for example, the relative flux

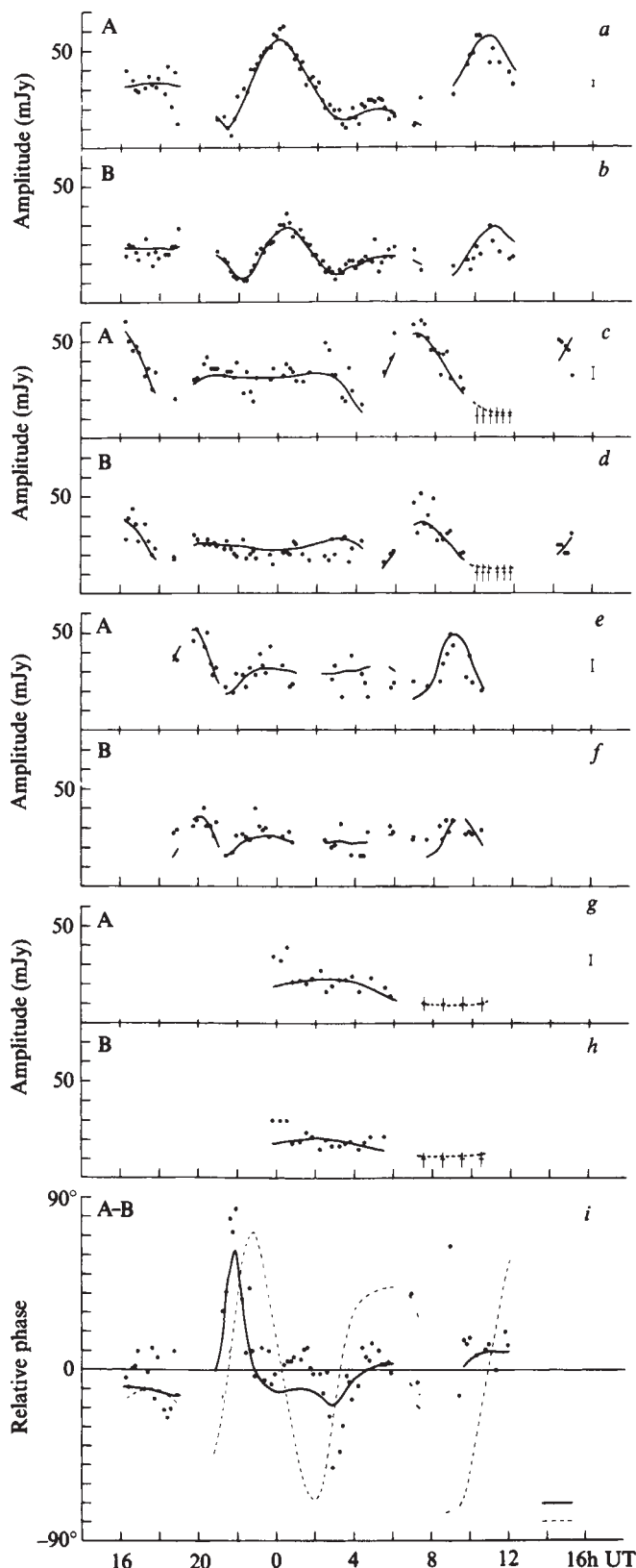


Fig. 1 a–h, Amplitudes of the visibility functions of components A and B at 1,666 MHz on the following baselines: a, b, Effelsberg–Jodrell Bank; c, d, Onsala–Effelsberg; e, f, Onsala–Jodrell Bank; g, h, Effelsberg–Green Bank. The solid line through the points represents the two-component gaussian models whose parameters are given in Table 1. 1σ error bars are shown in the top right corners. i, Relative phase of the visibility functions (component A–component B) at 1,666 MHz on the baseline Effelsberg–Jodrell Bank. The solid line represents the relative phase predicted from the models fitted to the amplitude data for similarly oriented structures; the broken line is that predicted for opposite orientations. (At 0 h UT, Greenwich ST = 10h 08min.)

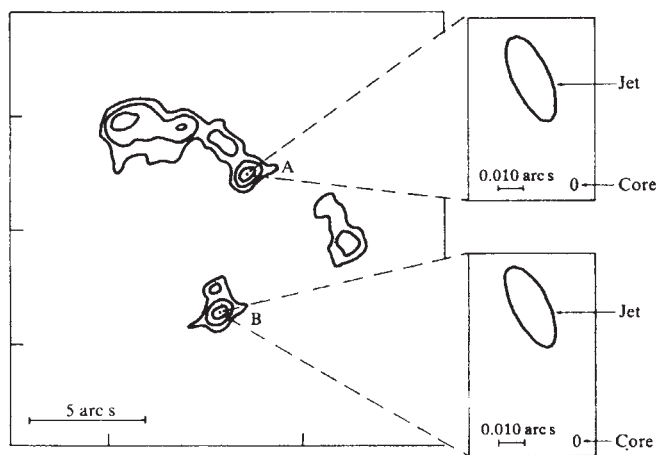


Fig. 2 Outline of the VLBI structures of the images A and B (half-power contour of the fitted gaussian models) projected against the VLA map of Greenfield *et al.*¹².

density between the cores and jets, and their sizes. We have further constrained the flux densities of the jets, however, by requiring that the total model flux density for each component does not exceed the corresponding total flux density derived from low-resolution maps. For this purpose we used the spectra given by Noble and Walsh¹³ derived from published flux density values at various frequencies. This procedure seems appropriate as the maximum correlated flux densities we obtained equal these derived values (61 mJy and 43 mJy, respectively).

In general we have only used data from integration periods where we have unambiguously detected both A and B. Occasionally, however, both are undetected, in particular during a 4-h period at the end of the track on the Effelsberg–Green Bank baseline. We have used this information to constrain the models to give low values in these periods and its main effect is to require a slight extension of the core components of ~ 3 marc s in PA $\sim -10^\circ$. One region where our gaussian model parametrization of the structures is not quite adequate is at the beginning of the Effelsberg–Green Bank track. This indicates the existence of an additional fine-scale structure not represented by these simple core–jet models.

Model fitting to only the amplitudes of the measured visibility functions leads to a 180° ambiguity in the orientations of the structures in both A and B. To resolve this ambiguity we have analysed the relative visibility phase between components A and B on the baseline Effelsberg–Jodrell, which has the highest signal-to-noise ratio (and hence smallest phase noise errors). As the components A and B are separated by only ~ 6 arc s, measurements of their relative phase are effectively uncorrupted by the various perturbations caused by the atmosphere and the equipment. The relative phase, therefore, represents the true difference between the phase of the visibility functions of A and B. This itself is composed of (1) a sinusoidal function of period 24 h representing the position difference between A and B, and (2) other terms representing any difference in their structure.

We first removed from the measured phase difference a term corresponding to the centroid separation measured by Porcas *et al.*¹⁰. The correct values are $\Delta RA = -0.1499$ s, $\Delta dec = +6.047$ arc s. Due to an algebraic error, the differential precession correction was wrongly applied for the right ascension difference quoted in our previous paper. The corrected values agree well with those quoted by Gorenstein *et al.*¹⁹ from high-sensitivity transatlantic VLBI measurements at 2.3 GHz. The residual phase difference, representing the difference in the structures, is shown in Fig. 1i.

In interpreting Fig. 1i we consider the following points. If the structures of A and B were identical and oriented in the same sense, the difference phase would be everywhere zero. If A and B had oppositely directed structures then, as the individual

structures are themselves asymmetric, the relative phase would reflect this asymmetry, giving values twice that of the phase of either individual visibility function. We assume that the measured centroid separation in fact corresponds to that of the core components in A and B. We would then expect to see these phase departures occurring at hour angles where the jet component (which is 'resolved out' at most hour angles where the projected baseline is not perpendicular to the jet) starts contributing to the correlated flux density.

For the case we have here, the structures of A and B differ anyway, primarily in the separations between the core and jet. Thus, even in the case of the same orientation, we expect imperfect cancellation of the visibility phases where the visibility functions differ most, that is near the minima. This is indeed apparent from Fig. 1i, where there are rapid relative phase excursions at UT 22h and 03h. Of particular interest, however, is the return of the relative phase to near zero immediately after the 22h excursion and lasting until the 03h excursion. This is precisely what would be expected for similar orientations as, when both jets contribute to the correlated flux densities, the centroid separation will be similar to that of the cores. (Indeed, this property has enabled us to deduce the correct centroid separation from the consistent phase behaviour away from the minima in our 1979 data.) This is not true for oppositely oriented structures.

This agreement is confirmed by examination of the relative phase predicted from the models of the A and B structures fitted to the amplitude data. The solid curve in Fig. 1i is that for similar orientation of structures, with both A and B jets pointing to the north-east; the dashed curve is that for opposite orientations. Although the models do not accurately reproduce the depth of the phase excursion, the gross features of the measured phase difference agree well for the case of the same orientation.

Table 1 Gaussian model parameters

	Separation (marc s)	PA	Flux density (mJy)	Maj. axis (marc s)	Axial ratio	PA
Image A						
Core	—	—	29	3.5 $\pm$ 1.5	0.5 $\pm$ 0.1	$-10^\circ \pm 6^\circ$
Jet	46 $\pm$ 1	21 $^\circ \pm 1^\circ$	32	38 $\pm$ 3	0.35 $\pm$ 0.04	27 $^\circ \pm 3^\circ$
Image B						
Core	—	—	24	2.5 $\pm$ 2.7	0.5 $\pm$ 0.2	$-7^\circ \pm 15^\circ$
Jet	56 $\pm$ 2	17 $^\circ \pm 1^\circ$	17	37 $\pm$ 6	0.36 $\pm$ 0.1	28 $^\circ \pm 3^\circ$

A more indirect way of obtaining the component orientations individually uses the 'closure' phase, that is the algebraic sum of three simultaneously measured visibility phases around a closed triangle of baselines¹⁸. However, to obtain useful closure phase information the data must have a good signal-to-noise ratio on all three baselines for a sufficient period. This was not the case for our data for not only are those from the Onsala baselines very noisy but also there is only one period, from 21.30 to 00.40 UT, where we have reliably separated the contributions from A and B on all three baselines simultaneously. The closure phases for this 3-h period do show features corresponding to the rapid phase changes occurring on the Effelsberg–Jodrell baseline during this time; however, although these are consistent with our deductions from the relative phase, the closure phases do not in themselves provide a reliable independent check on the component orientations.

Our highest-resolution data were obtained from the Effelsberg–Green Bank baseline at 4,996 MHz, with minimum lobe spacing of 2 marc s. With a 20-min integration time we obtained an r.m.s. noise level of 8 mJy, and for much of the track we detected both A and B components at levels of ~ 28 and ~ 25 mJy respectively, indicating very compact (≤ 1 marc s) structure in the cores. For the later part of the track, however, both components get weaker and fall below our detection limit. This suggests that the compact core components of A and B are

slightly elongated at 4,996 MHz in the same direction as at 1,666 MHz. Haschick *et al.*¹⁴ have obtained very similar data at 5,010 MHz on this baseline, although they attribute the non-detection of A and B at the end of the track to telescope pointing errors.

The agreement of the flux densities detected at the start of the track with the total 5-GHz flux densities of A and B shown by the lower-resolution maps^{8,9,11,12} suggests that the jets seen at 1,666 MHz are much weaker at 5 GHz and thus have a steep spectrum.

Interpretation and discussion

The similarity of the structures of A and B clearly confirms that we are seeing two images of a single structure. The core-jet configuration seen in each image is typical of the compact structures of many QSOs and radio galaxies²⁰. Figure 2 shows an outline of the model VLBI structures for A and B projected against a map of the larger-scale structure. In the lower-resolution maps at 5 GHz (ref. 12) and 408 MHz (ref. 13) there is an extended bridge of emission between A and the outermost extended structure to the north-east. The jet in the VLBI structure does not point directly at this bridge but it is possible to draw a smooth curve from the core of A through the jet and along the bridge suggesting a continuity between these features. Young *et al.*⁴ suggest that the curvature of the complex consisting of A together with the extended structure to the north-east and to the west may be the result of gravitational imaging of a linear structure. The curvature implied by the VLBI structure seems too extreme for this explanation, and the structure of the object itself is probably non-linear. In fact, if component B is excluded, the overall structure is rather similar to that of the QSO 3C309.1 (ref. 21) which is also a triple source showing a marked rotation in position angle between the nucleus and the outer lobes.

The details of the VLBI structure place severe constraints on the mass distribution of the lens. The results of Young *et al.*³ show that the previously observed properties of the double QSO cannot be produced by a single spherically symmetric mass distribution; such a distribution would require A, B and the galaxy G1 to be co-linear as opposed to the observed skew image line. In their investigation of mass distributions to account for previous observations, Young *et al.*⁴ "found it remarkably difficult to fit both the skew in the line and the high brightness of

B relative to A. Fitting either one is reasonably easy, but fitting both strained the capacity of the present models".

Paradoxically the close similarity between the image structures derived from our VLBI observations is most surprising. To see why this should be so, it is instructive to compare them with those expected with a spherical mass distribution. Bourassa and Kantowski²² showed that a spheroidal mass distribution will produce one or three images (or, more generally, an odd number). Thus we expect at least one additional image besides A and B. This has been discussed in the context of 0957+561 (refs 3, 23) and it is expected that the B image may be split into two separate images. We adopt the notation of Young *et al.*³ and refer to these as B2 (the outermost image) and B1. Figure 3 illustrates the imaging of an element of the object. We can confidently identify our B image with B2 since the jets in both A and B components point north. Two properties of our model that are quite different from what is produced by a spherical mass distribution are (1) the relative PAs of the core-jet lines, and (2) the relative linear dimensions of the two images.

(1) With a spherical distribution the PAs of the core-jet lines should have opposite signs with respect to the line joining the two images and the galaxy. In our model this is not so; in fact the two lines are almost parallel. Referring to the actual skew galaxy-image configuration³, the core-jet line of image A has PA +37° with respect to the line A-G1, and the core-jet line of image B has PA +6° with respect to the line B-G1. (We use Stockton's position⁵ for G1, that is, 0.19°E and 1.00°N of B.) The fact that the line of structure B points almost directly at G1 must be attributed to chance, because it is determined by the corresponding line of the object which must have a random orientation if the gravitational imaging interpretation is correct. (2) Because the surface brightness of the images and object must be the same, the flux density ratios of the two images produced by a spherical mass distribution is

$$\frac{S_A}{S_B} = \left| \frac{\theta_A}{\theta_B} \cdot \frac{\Delta\theta_A}{\Delta\theta_B} \right|$$

The ratio of the total flux densities is S_A/S_B is 1.42. (The ratio from our models is 1.9 for the jets and 1.2 for the cores. Though differential amplification is possible along the lengths of the images, these observed values are subject to the uncertain division of flux between core and jet in each component, as pointed out earlier; they cannot be regarded as significantly different.) The observed ratio $|\theta_A/\theta_B|=5$, so the value of $|\Delta\theta_B/\Delta\theta_A|$ from the above formula is 3.5. The best estimate of $|\Delta\theta_B/\Delta\theta_A|$ from our model comes from the core-jet separations and is 1.2 (this is confirmed by the major axes of the jets, for which a value 1.0 is obtained; taking into account the errors, this is not significantly different from 1.2). Thus the length ratio of the two images differs by a factor of 3 from that expected from the simple case of a spherical mass distribution.

It is of interest to put limits on the intensity of image B1. Our visibility functions for B are sensitive to structure on scales up to ~200 mas. From the lack of any further modulation on our best defined visibility function (Effelsberg-Jodrell Bank) we can put an upper limit of ~5 mJy on the flux density of B1. This rules out Young *et al.*'s³ model (c) for the system. For separations of B1 from B2 greater than this we become increasingly less sensitive due to smearing of the visibility function by our 10-min integration time, but the VLA map of Greenfield *et al.*¹² already puts strong limits on B1 in this angular range. There is, however, a weak compact feature on the VLA map, coincident with the galaxy G1 within the observational errors, and it has been suggested that this is either the image B1 or emission from G1 itself. This feature is too weak to be detected by our observations, so we cannot say whether it, too, is compact like the A and B components.

Finally, we consider the effect of any intrinsic variations in the radio and optical emission from the quasar. The estimation of the time delay in the variation between images A and B depends on detailed modelling of the lens galaxy and the surrounding cluster^{3,4,24}. Variations of the relative intensity in the optical

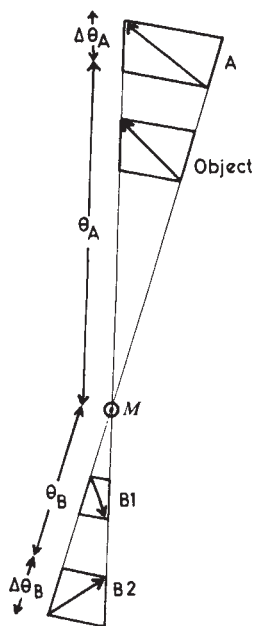


Fig. 3 Schematic representation of the imaging of an object element by a mass distribution, M , which has spherical symmetry. The arrows indicate an arbitrary line in the object and the images of this line.

continuum emission have already been reported^{4,25} and also in the 5-GHz radio emission¹² and the UV continuum (R. Wilson, personal communication). At frequencies of 1.6 GHz and lower much of the radio emission comes from the steep spectrum jet. Because this is extended and lies ~ 100 pc away from the core component, we cannot expect this radio emission to vary on time scales as short as a few years. We would thus expect the ratio of the intensities of A and B to vary less at lower frequencies.

Our VLBI measurements of the structures of A and B therefore confirm the interpretation of 0957+561 as a QSO seen through a gravitational lens. The intrinsic radio structure of the QSO has two outer lobes and a compact central component consisting of a jet and a core. We have put limits on the

properties of any third image which an extended, transparent lens is expected to produce. The surprisingly close similarity of the orientations of the images and their sizes must be attributed to the non-spherically symmetric elements of the imaging mass distribution.

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Halogen and phosphorus storage in the Earth

J. V. Smith

Department of the Geophysical Sciences, University of Chicago, Chicago, Illinois 60637, USA

Speculations on the spatial distribution of elements involve geochemical, geophysical and cosmochemical factors. From analyses of basalts and minerals in peridotites, it is found that: apatite with ~ 3 wt% F, and up to 1 wt% Cl and 0.003 wt% Br, is the principal reservoir of halogens in the upper mantle and mica with ~ 0.5 wt% F and 0.05 wt% Cl is a second reservoir; apatite with ~ 18 wt% P accounts for most of the phosphorus in the mantle, but is insignificant with respect to ~ 0.7 wt% P in the core; surface reservoirs contain at least two-thirds to three-quarters of the Cl and Br, but only one-fifth of the F and $<1\%$ of the P and finally the Cl and Br contents of the whole Earth are $<66 \times 10^{18}$ and $<17 \times 10^{16}$ kg, using a new estimate of $\sim 10 \times 10^{19}$ kg F.

ALL cosmochemical models for the Earth involve less efficient capture of volatile elements than refractory ones from the solar nebula, but accretion processes¹ are complex. The controversial Ganapathy-Anders^{2,3} (GA) model relates elements that would condense nearly together during simple progressive cooling under equilibrium. Chemical analyses of surface reservoirs, coupled with compositions for interior zones inferred from geophysical and geochemical data, were used to obtain a range of estimates of the bulk composition of the Earth⁴. Particularly important are estimates of the extent of transfer of magmatophile elements from mantle to crust by partial melting, and back again by foundering⁵⁻⁷. In spite of uncertainties in assignment of elements to reservoirs a general correlation can be implied^{2,4,8-12} between volatility in the nebular gas and concentration in the whole Earth. Probable limits on the weight fractions of 14 to 20×10^{-9} U and 14 to 20×10^{-5} K are important.

Table 1 compares estimates of F, P, Cl, K and Br in near-surface reservoirs and whole-Earth models. The last row shows masses assuming that the bulk Earth has the same composition as carbonaceous meteorite of type C1. These masses are multiplied by 1.5 to account for overall depletion of volatile elements in the whole Earth (p. 351 of ref. 4). New analyses¹⁴ of halogens in C1 meteorites are lower than most earlier analyses¹³, especially for F, and a revised F/Cl ratio of 0.09 fits electron microprobe analyses of chlorapatites in meteorites (see ref. 15) and other bulk analyses^{16,17}.

Phosphorus should condense early in the 'metal' fraction of a nebular condensation model². Any uncertainty in the relative fractions of early condensate, silicate and metal components in the Earth is here considered trivial. Phosphorus is strongly bound in minerals of meteorites, and should not be lost to vapour during collisions of planetesimals. If P accretes with the same efficiency as Fe from a solar nebula with C1-type composition, its mass must lie between 10×10^{21} and 14×10^{21} kg, and probably between 11×10^{21} and 13×10^{21} kg.

Potassium and F fall into the volatiles 1,300–600 K group for which the depletion factor with respect to 1.5C1 composition can be estimated from radiogenic elements⁵. If the whole Earth contains between 140 and 200 p.p.m. K, and the revised K/F ratio in C1 meteorite is taken to be 9.1 from Table 1, the content of F should be between 9×10^{19} and 13×10^{19} kg. This gives 10×10^{19} for the GA model.

Cl and Br belong to the volatiles <600 K group. Although many mineralogical details do not fit the theory of equilibrium condensation¹⁸⁻²⁰, the concentrations of volatile elements in chondritic meteorites²¹ fit reasonably well with thermochemical calculations. Although similar depletion factors with respect to cosmic composition were obtained for Ti, Cl, Br and I in the bulk Earth by summation of Earth zones³, there is an uncertainty in the concentrations of these elements. There is evidence to show that peridotite xenoliths from the mantle were metasomatized by fluids, and the bulk analyses for volatile elements used in

Table 1 Reservoirs and chemical models

	F (kg)	P (kg)	Cl (kg)	K (kg)	Br (kg)
Hydrosphere	$19 \times 10^{14} \ddagger$	$13 \times 10^{13} \ddagger$	$27 \times 10^{18} \S$	$57 \times 10^{16} \ddagger$	$94 \times 10^{15} \S$
Sediments	—	—	$18 \times 10^{18} \S$	—	$10 \times 10^{15} \S$
Continental crust	} $18 \times 10^{18} \ddagger$	$29 \times 10^{18} \ddagger$	$23 \times 10^{17} \S$	$37 \times 10^{19} \ddagger$	$<1 \times 10^{16} \S$
Oceanic crust			$23 \times 10^{16} \S$		$82 \times 10^{14} \S$
Total	18×10^{18}	$29 \times 10^{18} \ddagger$	$48 \times 10^{18} \S$	$37 \times 10^{19} \ddagger$	$11 \times 10^{16} \S$
GA model, whole Earth ²	32×10^{19}	13×10^{21}	15×10^{19}	10×10^{20}	80×10^{16}
S2 model, whole Earth ¹	54×10^{19}	11×10^{21}	27×10^{19}	78×10^{19}	96×10^{16}
Sh model, whole Earth ¹²	29×10^{19}	—	24×10^{19}	19×10^{20}	—
1.5 × Cl composition, revised*	53×10^{19}	99×10^{20}	62×10^{20}	48×10^{20}	29×10^{18}
GA model, revised†	10×10^{19}	—	12×10^{19}	—	55×10^{16}

*P and K from ref. 4 Table 2, col. 1; F, Cl and Br from ref. 14 Table 3, row 7.

†Adjusted by ratio of abundances in ref. 13, Table 4, rows 1 and 8.

‡ From ref. 4.

§ From ref. 10.

ref. 4 are higher than recent ones for selected xenoliths²². Nevertheless, the depletion factors for this group of volatile elements must be assumed to be constant. Ganapathy and Anders² modelled the group from an observed Ti/U ratio of 0.27 in terrestrial rocks and an assumed U content of 18 p.p.b. (parts per 10⁹) in the whole Earth. Because the measured Ti/U ratio of 7.0 (atomic) for carbonaceous chondrites²³ is close to the value of 7.3 chosen by Cameron¹³ for the cosmic composition, and the assumed U content² of 18 p.p.b. for the whole Earth is near the middle of the range 14–20 p.p.b. assumed here, the Ti and U content of the GA model needs no adjustment. However, revised estimates of Cl and Br are given.

Potential mineral hosts

Systematic electron microprobe analyses were made of P, F and Cl in minerals from the mantle^{24,25}. Halogens were detected only in mica, amphibole and apatite. Amphibole is restricted to the outer 100 km: MARID-suite and secondary-textured crystals in peridotite xenoliths, F 0.5–1.0, Cl 0.00–0.03, K 0.3–4.3 wt%; kaersutite megacrysts in alkali basalts²⁶, F 0.07–0.22, Cl 0.001–0.012, K 1.0–1.7 wt%. Phlogopite-rich mica is stable down to ~200 km, with the following averages: primary texture in depleted peridotites, F 0.43, Cl 0.08, K 8.6 wt%; secondary textures of various types in peridotites, F 0.63, Cl 0.03, K 8.6 wt%. No large apatite crystals in textural equilibrium with silicate minerals of peridotite xenoliths in kimberlites have been found, but apatites from two lherzolite inclusions in basanites²⁷ have 0.9 and 1.0 wt% Cl. Apatites²⁸ from apatite-rich xenoliths in basalts from eastern Australia²⁹ show 2.1–2.7 wt% F and 0.17–0.22 wt% Cl. A small euhedral crystal enclosed in a dolomite bleb, itself enclosed in a Cr-diopside megacryst found in a kimberlite, contains 0.9 wt% Cl and 1.4 wt% F (ref. 30). A megacryst from the Moses Rock 'kimberlite' contains 0.5 wt% F and <100 p.p.m. Cl (ref. 25). Apatites from seven carbonates²⁵ contain high F (2.0–3.7 wt%) and low Cl (0.00–0.1 wt%), and all but one apatite grain from serpentine veinlets in peridotite xenoliths from kimberlites contain low Cl. All apatites contain ~40 wt% P₂O₅, and all silicate minerals contain little P₂O₅, mostly below 0.03 wt% (ref. 24).

Complete incorporation of P into melt was adopted for a model of basalt petrogenesis from melting of mantle pyrolite³¹. A strong correlation between Ce and P in a wide range of basaltic rocks^{31,32} can be explained either by the presence of most of the Ce and P in apatite which was totally consumed during melting, or by similar bulk solid-liquid partition coefficients for Ce and P (ref. 32). The good correlation between F and P in oceanic basalts and gabbros suggests that these two elements were present mainly in apatite²⁵, and rules out the occurrence of whitlockite unless it survived the generation of basalt. Total consumption of apatite is consistent with experiments on apatite saturation in magmas at high pressures³³.

Analyses of F, P, Cl and K in pillow interiors of tholeiitic basalts from the Mid-Atlantic Ridge³⁴, alkali basalts from the Azores Islands³⁴ and tholeiitic basalt from the East Scotia Sea back-arc basin³⁵ were used (Table 2) to determine whether these elements were derived solely from total incorporation of F, Cl-bearing mica and apatite into melt. The atomic ratio (F+Cl)/P for hydroxyl-free apatite is one-third. As all rocks in Table 2 have an average ratio higher than one-third, some halogens must be derived from mica. Table 2b shows the amounts of F and Cl needed if K were attributed to mica with the mean composition of the primary-textured type in depleted peridotite xenoliths transported by kimberlites. For the normal mid-ocean ridge basalts and the back-arc-basin basalts, the remaining F and Cl yield (F+Cl)/P ratios close to one-third. For the alkali basalts, the ratio drops to 0.08, which might be explained by: hydroxyl in apatite; lower halogen content of the mica; and derivation of K from halogen-free clinopyroxene³⁶. The final option can be ruled out because the K content is too high (12,000 p.p.m.) with respect to the Ca content (~6 wt%). Only the basalts from the Azores transect have a (F+Cl)/P ratio too high to be attributed to hydroxyl-free apatite and primary-type mica, and there are at least three explanations: Cl-metasomatism by seawater; higher halogen content of mica; and precipitation of a mineral with high K/(F+Cl) in a sub-surface magma chamber.

It is assumed here that (1) for tholeiitic basalts from normal ridge segments, assignment of one-fifth of the K to clinopyroxene and four-fifths to primary-type mica would allow the apatite to contain 0.87 atoms of F and 0.13 atoms of Cl; (2) for the back-arc-basin basalts, the apatite contains about 0.67 atoms of F and 0.33 atoms of Cl; and (3) for the alkali basalts, the apatite contains about 0.75 atom of F and 0.25 atom of Cl, while the source mica has ~0.15 wt% F.

Complex processes should be expected however, during transport of a magma to the surface. Tholeiitic basalts at mid-ocean ridges are probably the residue of crystal-liquid processes in near-surface magma chambers³⁷, and crystallization of apatite and amphibole may cause complications. Apatites from altered gabbros and troctolites from the Mid-Cayman Rise contain 0.15–0.3 wt% Cl (no analysis for F), and most amphiboles contain little Cl (~0.01 wt%) while a few contain substantial amounts (up to 0.9 wt%)³⁸. Because F enters crystals in preference to melt, surviving basaltic melt should lose F with respect to parent melt. It will now be assumed that F, Cl-rich apatite and F, Cl-bearing mica are the principal hosts for halogens and phosphorus in the upper mantle. This is consistent both with mass-balance calculations for two lherzolite xenoliths²⁴ which indicate that silicate minerals provide only about one-tenth of the phosphorus, and with the expectation that H₂O should fractionate preferentially into magma over apatite during crystal-liquid differentiation of the Earth, and the low abundance of primary water in mid-ocean-ridge tholeiitic basalts³⁹.

Table 2 Interpretation of F, P, Cl and K analyses of pillow interiors of oceanic basalts (A–C) and glassy pillow rims of back-arc-basin basalts (D)**a** Bulk analyses and atomic ratios

Type	Weight: mean and 1 σ				Atomic ratios		
	F (p.p.m.)	P (p.p.m.)	Cl (p.p.m.)	K (p.p.m.)	C/P	Cl/P	(F + Cl)/P
A ³⁴	389 $\pm$ 131	1,110 $\pm$ 389	454 $\pm$ 221	5,230 $\pm$ 2,302	0.571	0.357	0.928
B ³⁴	144 $\pm$ 28	605 $\pm$ 156	38 $\pm$ 14	940 $\pm$ 470	0.388	0.055	0.443
C ³⁴	586 $\pm$ 118	2,441 $\pm$ 517	350 $\pm$ 210	12,000 $\pm$ 3,900	0.391	0.125	0.516
D ³⁵	252	794	124	2922	0.517	0.136	0.653

b Calculated values assuming primary mica in source region

Type	F(mica)	Cl(mica)	F(residual)	Cl(residual)	F/P(res.)	Cl/P(res.)	(F + Cl)/P
A	261	49	128	405	0.188	0.319	0.507
B	47	9	97	29	0.261	0.042	0.303
C	600	112	-14	238	-0.009	0.085	(0.085)
D	146	27	106	97	0.217	0.106	0.323

A 20 Mid-Atlantic Ridge tholeiitic basalts from Azores transect (37–40°N).

B 16 Mid-Atlantic Ridge tholeiitic basalts from two normal ridge segments 28–33 and 49–52°N.

C 18 alkali basalts from the Azores Islands.

D 5 back-arc-basin basalts from East Scotia Sea.

Because Cl/Br ratios range only from 222 to 494 for rocks from oceanic ridges and islands^{10,40}, Br must have the same mineral hosts as Cl.

Tholeiitic basalts from mid-ocean ridges are more abundant than alkali basalts from oceanic islands and the diverse range of magmas in continents, and perhaps give the best clue to the average composition of the upper mantle. Although the compositions of the mineral hosts for halogens and phosphorus cannot be determined, it is suggested that the source regions of basalts contain F, Cl-rich apatite. Values of 3 wt% F and 1 wt% Cl are used for the initial calculations. Because electron microprobe analyses of Cl in apatite from upper-mantle xenoliths range from 0.2 to 1.0 wt% the effect of reducing the Cl content to 0.3 wt% will be considered. For mica, rounded values of 0.5 and 0.05 wt% will be assumed for F and Cl, respectively.

Estimate of P distribution

Subtraction of 18×10^{18} kg F and 37×10^{19} kg K for the crust from whole-Earth estimates in the revised GA model of 10×10^{19} kg F and 10×10^{20} kg K leaves 8×10^{19} kg F and 6×10^{20} kg K for the mantle. Assignment of all K to mica with 8.6 wt% K and 0.5 wt% F would require 35×10^{18} kg F. For apatite with 3 wt% F and 18 wt% P, the remaining 45×10^{18} kg F would require 27×10^{19} P. This is a small fraction of the estimated 13×10^{21} kg P in the whole Earth and hence details of mineral compositions can be ignored. Whitlockite, $\text{Ca}_3(\text{PO}_4)_2$, was ruled out as a mineral host for P in the upper mantle. The crystal structure of apatite will be assumed to be stable to high pressures. Hence it is proposed that the remaining P goes either into silicate minerals in the deep-mantle or into the core. Because the mass of the entire mantle is 40×10^{23} kg, a mean concentration of 0.3 wt% P would be required for the silicates. Although this is >10 times greater than the concentrations found in olivine, pyroxene and garnet minerals of peridotite xenoliths²⁴, this possibility should not be ruled out. Experimental syntheses on garnet⁴³ should be extended to higher pressure. The P could be assigned to the core, for which a concentration of 0.7 wt% would be required. This value lies inside the range for iron meteorites⁴¹ (type I 0.2 wt%; type II 0.12–1.0 wt%; type III 0.07–1.2 wt%), and is consistent with Earth models based on analogy with meteorites. Assigning 0.03 wt% P to the average mantle would lower the estimate of 0.7 wt% in the core to 0.6 wt%. High-pressure reduction of phosphate to phosphide during Earth accretion should also be considered. The inferred content of 0.7 wt% P in the Earth's core is much less than the ~10 wt% S (ref. 42) required to lower the density of the outer core from that for liquid Fe, Ni and there seems to be no geophysical test of the proposed content of P. Proposed contents of C and N in the core⁴ are less than for P, but also amount to a substantial fraction of whole-Earth estimates.

Estimate of Cl and Br distribution

Subtraction of the estimate of K in the crust (37×10^{19} kg) from the estimated value for the whole Earth (10×10^{20} kg) leaves 6×10^{20} kg for subcrustal regions. Assigning all this K into primary-textured mica of depleted peridotite would produce 5×10^{18} kg Cl and into secondary-textured mica 2×10^{18} kg. If the mass of F in apatite of the Earth's mantle is 45×10^{18} kg, and the apatite contains 3 wt% F and 1 wt% Cl, the mass of Cl is 15×10^{18} kg. Reduction of the Cl content of apatite to 0.3 wt% drops this mass to 5×10^{18} kg. Hence apatite seems to be a more important store of Cl in the mantle than mica, and especially than amphibole. The estimated mass of $17\text{--}20 \times 10^{18}$ kg Cl in the mantle (for apatite with 1 wt% Cl) implies that 25–30% of the Earth's chlorine remains in the mantle. The estimated mass of $7\text{--}10 \times 10^{18}$ kg Cl in the mantle (for apatite with 0.3 wt% Cl) implies that only one-seventh of the Cl remains in the mantle. The $65\text{--}68 \times 10^{18}$ kg and $55\text{--}58 \times 10^{18}$ kg are about half of the estimated whole-Earth content of 12×10^{19} kg in the revised GA model.

For Br in the mantle, a Cl/Br ratio of 300, taken from the range of 222–494 in oceanic basalts^{10,40}, leads to estimates of 6×10^{16} and 3×10^{16} kg Br, depending on the assumed Cl content of apatite. These values are about one-half and one-quarter of the estimate of 11×10^{16} kg in the crust and hydrosphere¹⁰, and imply that about one-quarter to one-third of the Earth's Br remains in the mantle. The $14\text{--}17 \times 10^{16}$ kg is about one-third of the estimated whole-Earth value of 55×10^{16} kg in the revised GA model.

The above conclusions assume that halogens are absent from the principal minerals of the Earth's mantle. To bring the $65\text{--}68 \times 10^{18}$ kg Cl up to 12×10^{19} kg Cl in a revised GA model would require $\sim 13 \times 10^{-6}$ weight fraction throughout the entire 40×10^{23} kg of the mantle; from general crystal-chemical considerations this does not seem plausible.

Conclusions

Within the many uncertainties, I suggest (1) apatite with ~3 wt% F, up to 1 wt% Cl and 0.003 wt% Br is the principal mineral reservoir for halogens, and mica is a subsidiary reservoir; (2) apatite with ~18 wt% P is the principal store of P in the upper mantle and perhaps the lower mantle, but accounts for only one-twentieth of P in the Earth; (3) the remaining P is in a reservoir inaccessible to magmatism, and may amount to a maximum of 0.7 wt% in the core; (4) at least two-thirds to three-quarters of the Cl and Br have entered the crust, but only one-fifth of the F (Table 1) and 0.2% of the P; (5) the whole-Earth contents of halogens, K and P are consistent with less efficient capture of volatile elements than refractory ones from the solar nebula; and (6) the Cl and Br contents should be reduced in the GA model. The distribution of halogens is

relevant to crystal-liquid differentiation of the Earth. If all Cl in a subducted slab is returned to the surface by island-arc volcanism^{38,44,45}, about one-third of the Cl in the mantle has not been transported to the surface, at least since the onset of subduction in the era of plate tectonics. If Cl was returned from the crust to the mantle during the turbulent conditions of the pre-Archaeon era⁷, it would not be necessary to argue that one-third of the Earth did not melt during accretion. Because present data on phase equilibria indicate that both apatite³³ and hydroxylated silicates⁴⁶ are consumed early by partial melting, it would be difficult to justify a model in which halogens could be subducted in preference to K and Rb. Simple two- or three-reservoir models^{5,6} for radiogenic elements suggest that the Earth's crust was extracted from only one-half to one-third of the mantle, assuming that the original mantle was chemically

uniform (see also ref. 47). This assumption of chemical uniformity may be incorrect, especially for the halogens. It is difficult to see how apatite could have been distributed uniformly throughout the mantle during accretion in view of the large amounts of accretional and core-formation heats. The possibility that apatite in the mantle tended with time to become more refractory by preferential loss of Cl, Br and OH to magma should be considered. Therefore considerable study of geochemical models for the origin and differentiation of the Earth, and further measurements of trace elements in mantle minerals are needed.

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Population dynamics of fox rabies in Europe

Roy M. Anderson*, Helen C. Jackson†, Robert M. May‡ & Anthony M. Smith*

*Zoology Department and Centre for Environmental Technology, Imperial College, London University, London SW7 2AZ, UK
†WHO Collaborating Centre for Rabies Surveillance and Research, Federal Research Institute for Animal Virus Diseases, D7400 Tübingen, Postfach 1149, FRG

‡Biology Department, Princeton University, Princeton, New Jersey 08450, USA

A simple mathematical model for the overall dynamics of the interaction between fox populations and rabies is presented. The model helps to explain epidemiological patterns observed in Europe, including the 3 to 5 year cycle in fox populations infected with rabies, threshold densities and average levels of prevalence of infection. We give a quantitative discussion of the possibilities of controlling rabies by culling or vaccinating foxes (or by a mixture of the two).

RABIES is a directly transmitted viral infection of the central nervous system, to which all mammals are thought to be susceptible (although not equally so)¹⁻⁴. Throughout human history it has been one of the most feared of all infectious diseases^{1,5-9}. Today, the number of people dying from rabies in Europe is insignificant (around 4 per annum in recent years), and even the global total (estimated as high as 15,000 per annum, mainly in India)¹⁰ is dwarfed by other diseases. However, the nature of the clinical symptoms, which are distressing and often hideous, plus the knowledge that death is virtually inevitable once symptoms appear^{8,11}, makes rabies a continuing cause for concern.

Many major epidemics of rabies have been recorded in Europe over the past seven centuries^{1,8}, with the dog acting as the main host and primary transmitter of the infection to man. The current epidemic in Central Europe is thought to have originated in Poland in 1939 (ref. 12), and is characterized by a

high incidence in the red fox *Vulpes vulpes*; of the 16,820 cases of animal rabies reported in Europe in 1979, over 70% were in this host species¹³. Throughout Europe, and to a lesser extent in Canada and the United States, foxes (red, grey and arctic species) play a dominant role in the transmission and maintenance of the disease, although other mammals (including dogs, skunks, badgers, raccoons, wolves and bats) are important in these and other regions of the world^{1,7,14-17}.

The current European epidemic has spread outwards from Poland at an annual rate of 30-60 km, with the present front in France being less than 40 km from the English Channel coastline (Fig. 1). The introduction of rabies into Britain through illegally imported pets¹⁸ therefore seems a distinct possibility and is especially worrying because of the uniquely high urban and suburban density of foxes, side by side with dense populations of dogs and cats^{19,20}.

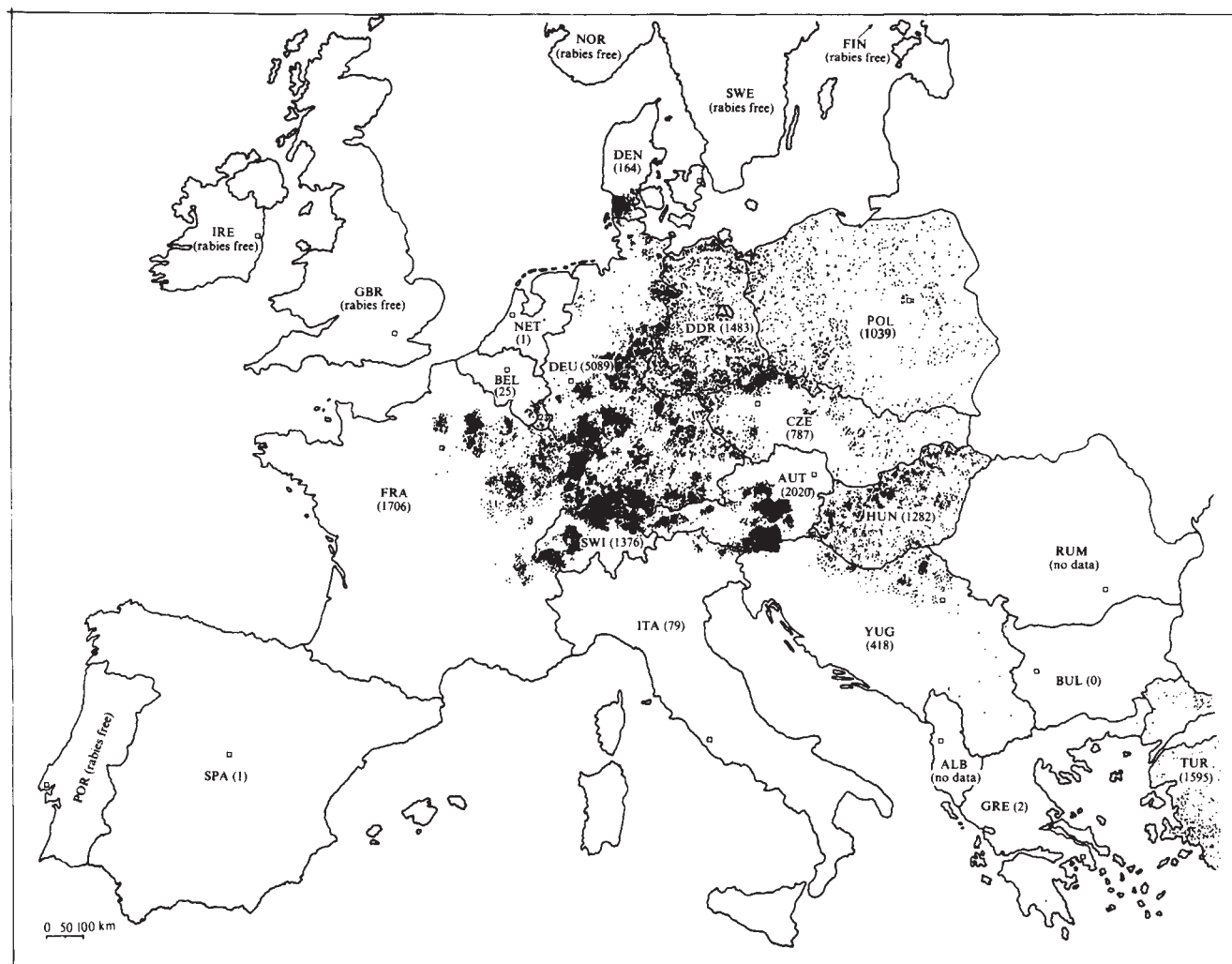


Fig. 1 Geographical distribution of reported European cases of rabies, both in wildlife and domestic animals, during 1979. Each dot denotes one reported case, excepting a few dots in northern Switzerland and southern Germany which represent between one and four cases.

Previous studies of the epidemiology of rabies in fox populations have focused on the characteristics of the viral infection within the host^{1,14,21-26}, on the ecology of the fox^{14,18-20,27-33,101} and on computer simulations along with statistical studies of the spatial and temporal development of epidemics³⁴⁻⁴³. This article, however, takes a different approach in studying the overall population biology of the interaction between foxes and rabies with the emphasis on recurrent epidemic behaviour (in contrast to the more usually studied spatial aspects of rabies epidemiology). We present a simple mathematical model which exhibits patterns of dynamical behaviour in striking agreement with observed epidemiological facts. The model enables us to identify the basic factors controlling persistence of the pathogen, and to assess the effectiveness of different methods for controlling the disease.

Basic model and epidemiological parameters

We use a deterministic, compartmental model in which the fox population is divided into susceptibles, infecteds that are not yet infectious, and infectious individuals; these three classes are defined to have population densities X , I and Y , respectively^{44,45}. The model has no category of recovered, immune foxes, because few, if any, foxes recover once the virus is established in the host^{1-3,7,8,24,27}. The total density of foxes, N , is thus $N = X + I + Y$.

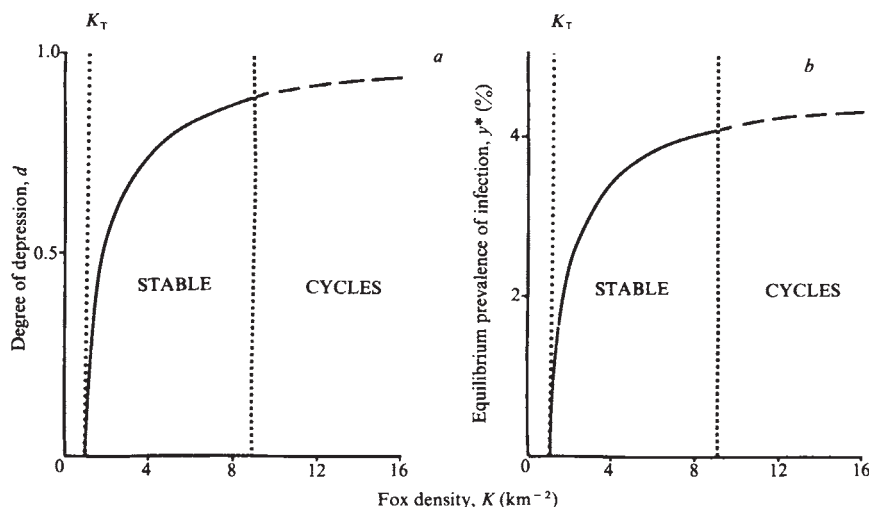
Foxes are territorial, a family's territory size depending on the type of habitat, food availability and the density of foxes^{19,20}. Home ranges in Europe and North America typically lie in the

range 2.5 to 16 km² (refs 46-52). In the absence of rabies, fox populations seem to increase up to some characteristic density, K , determined by the carrying capacity of the habitat. The value of K is set by the mixture of habitat types in a defined area¹⁹; within Europe, K is typically in the range 0.1-4 foxes per km² (refs 19, 20, 28, 52-54) although much higher densities have been reported in suburban and urban areas of Britain^{19,20}.

As the death rate of young foxes is density dependent^{19,20,28-32}, we assume the *per capita* death rate is linearly related to N , such that the net rate is $(b + \gamma N)N$; here, $1/b$ denotes fox life expectancy in the absence of resource limitation, and γ measures the severity of density-dependent constraints. Life expectancy, $1/b$, is typically in the range 1.5-2.7 yr within Europe (Table 1a); we take $1/b$ to be 2 yr (Table 1b).

Wandeler⁵⁵ argues that the rate of reproduction is independent of population density for foxes in Europe, although other reports suggest it may be limited by food availability^{30,31,53,54,101}. For simplicity, we assume that density dependence acts primarily on mortality, and that the *per capita* birth rate is a constant, a . The number of cubs in a litter ranges from 1 to 10, with a mean in Europe of 4.7 (refs 14, 18-20, 52, 55). Sex ratios are in general close to unity at birth, and the pregnancy rate is in the region of 90% (refs 19, 20, 55, 56) with a further 10% of vixens failing to produce offspring. By combining these values we obtain for a an average value of 1.0 per annum. The intrinsic *per capita* population growth rate, $r = a - b$, is then ≈ 0.5 per annum.

Fig. 2 *a*, Endemic rabies suppresses fox population density to a disease controlled equilibrium N^* where $N^* = K[(\sigma + a)(\alpha + a) - ar]/(\sigma\beta K - ar)$. This steady state density is necessarily less than the disease-free density K , and the degree of population depression induced by rabies, defined as $d = 1 - N^*/K$, is plotted for various values of K . In the region denoted 'stable' the model exhibits damped oscillations to a stable state, and in the region labelled 'cycles' limit cycle behaviour occurs and the dashed line denotes the average degree of depression throughout one cycle. Parameter values again are as listed in Table 1*b*. *b*, This graph portrays the relationship between the equilibrium prevalence of infection y^* and K , where $y^* = (Y^* + I^*)/N^*$, with Y^* and I^* the equilibrium densities of infectious and infected but not yet infectious (latent) foxes. By setting $dX/dt = dI/dt = dY/dt = 0$ in equations (2)–(4) in the text we obtain $y^* = [(\alpha + \sigma + a - v)/(\sigma + a)][r/(\alpha + a)]D$ where $r = r(1 - N^*/K)$, $D = [1 - 1/R]/[1 - (ar)/(\alpha + a)]$ and R is as defined in equation (5) of the text. The regions labelled 'stable' and 'cycles' are as defined in the legend to graph *a* and the dashed line in the cycles region denotes the average prevalence of rabies throughout one cycle. Note that both the degree of depression, d (graph *a*) and the prevalence y^* (graph *b*) rise as K increases.



These assumptions about the biology of the fox population lead to the familiar logistic equation⁵⁷ for the dynamics, in the absence of rabies:

$$dN/dt = rN(1 - N/K) \quad (1)$$

Here, the carrying capacity of the habitat at equilibrium is $K = r/\gamma$ (in practice, we try to estimate K itself, rather than γ).

Following conventional lines⁵⁸, we assume that the rate at which foxes acquire rabies is proportional to the number of encounters between susceptible and infectious foxes, βXY . Here, β is a transmission coefficient, with $1/\beta$ being proportional to the average time interval between fox contacts; reports suggest that individuals from adjacent family groups meet, on average, every 4 to 6 days²⁰. Foxes are assumed to pass from the latent or incubating class to the infectious state at a *per capita* rate σ , such that the average incubation period is $1/\sigma$. This period is variable for foxes (from 12 to 110 days) and depends on factors like age, site of infection and nutritional state⁵⁹; on average, $1/\sigma$ is around 28–30 days^{21–24}. Infectious or 'rabid' foxes have a short life expectancy, varying from 3 to 10 days, with an average of 5 days^{14,21–25}. We assume rabid animals die at a constant *per capita* rate α ; thus, $1/\alpha$ is their life expectancy.

Table 1 Life expectancy ($1/b$) of the red fox and values of epidemiological and other parameters

<i>a</i>	Life expectancy (yr)	Study area
	1.5	Denmark ⁹⁷
	1.7	Wales ¹⁸
	1.8	Scotland ²⁸
	1.9	Suburban London ⁹⁸
	2.2	Germany ⁹⁹
	2.7	Netherlands ¹⁰⁰

<i>b</i>	Symbol	Meaning	Value
<i>b</i>		Average <i>per capita</i> intrinsic death rate	0.5 yr ⁻¹
<i>a</i>		Average <i>per capita</i> birth rate of foxes	1 yr ⁻¹
<i>r</i>		<i>Per capita</i> population growth rate, $a - b$	0.5 yr ⁻¹
<i>K</i>		Fox carrying capacity, $K = r/\gamma$	Various
σ		$1/\sigma$ is the average latent period	13 yr ⁻¹
α		Death rate of 'rabid' foxes	73 yr ⁻¹
β		Transmission coefficient (estimated using equation (7)) for threshold density, $K_T = 1.0$ foxes per km ²	79.69 km ² yr ⁻¹

Because the infection is severely debilitating and of relatively short duration, we assume that infected foxes do not contribute to the reproductive effort of the population.

These biological assumptions lead to the following set of first order differential equations for the dynamics of fox populations infected with rabies:

$$dX/dt = rX - \gamma XN - \beta XY \quad (2)$$

$$dI/dt = \beta XY - (\sigma + b + \gamma N)I \quad (3)$$

$$dY/dt = \sigma I - (\alpha + b + \gamma N)Y \quad (4)$$

The equation for the total population is obtained by adding the above three:

$$dN/dt = aX - (b + \gamma N)N - \alpha Y \quad (5)$$

Table 1*b* summarizes our estimates of the values for the relevant parameters.

Population dynamics

The above formulation differs from conventional epidemiological models⁵⁸ in that the host population N is a dynamical variable, affected both by the disease and by density-dependent constraints imposed by resource limitation. Rabies will be maintained within the fox population provided the 'basic reproductive rate', R , of the infection exceeds unity^{60,61}; R is the expected number of secondary cases produced during the lifespan of an infectious fox introduced into a population of K susceptible animals. For the system defined by equations (2)–(4),

$$R = \frac{\sigma\beta K}{(\sigma + a)(\alpha + a)} \quad (6)$$

The condition $R > 1$ for endemic maintenance of rabies may equivalently be expressed as the requirement that the fox population exceed a 'threshold density'^{58,62}, $K > K_T$, with the definition

$$K_T = (\sigma + a)(\alpha + a)/\beta\sigma \quad (7)$$

If $R < 1$, or equivalently $K < K_T$, the fox population will settle to its disease-free equilibrium density K .

Epidemiological evidence suggests K_T lies in the range 0.25–1.0 foxes per km², with the most frequently reported value in Europe being around 1.0 foxes per km² (refs 18, 63–65). This observation can now be combined with equation (7), along with the parameter values listed in Table 1*b*, to give a rough estimate of the transmission parameter β . The value $\beta \approx 80$ yr (per unit density of foxes) thus obtained agrees remarkably well with independently obtained behavioural observations in rabies-free

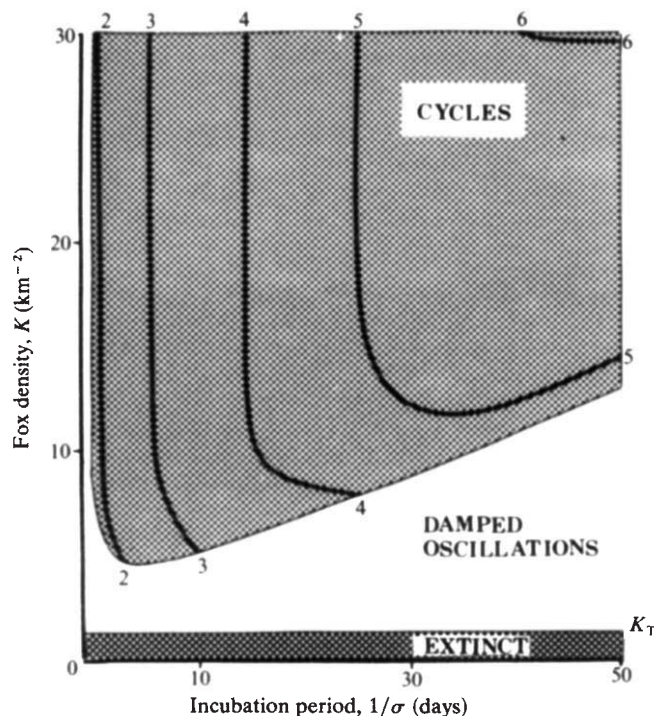


Fig. 3 The three regimes of dynamical behaviour that can be exhibited by the model defined in equations (2) to (4) are illustrated here in the $K - 1/\sigma$ parameter space. As discussed in the text these three regimes are: rabies unable to maintain itself within the fox population ($R < 1$), labelled 'extinct'; fox density controlled to stable equilibrium, N^* , by disease ($R > 1$), labelled 'damped oscillations'; and limit cycle behaviour in fox abundance and disease prevalence ($R > 1$), labelled 'cycles'. In this limit cycle region, the contour lines correspond to specific periods of oscillation, labelled in years. All rate parameters are expressed in units of yr^{-1} , and have the values listed in Table 1b.

populations concerning the average time interval between fox contacts in regions where the average density is around 1 fox per km^2 (which gives a value for $1/\beta$ of 4 or 5 days, or $\beta \approx 70\text{--}80^{-1}$ at this density)¹⁹. Such agreement seems surprising in view of the belief that rabid foxes have higher contact rates than do healthy animals, although altered host behaviour as a consequence of infection may act to compensate for the fact that only a certain proportion of contacts between healthy and rabid animals will result in disease transmission. When $R > 1$, rabies will regulate fox abundance below the disease-free level K (Fig. 2a). This regulated state, however, may be a stable constant value, or it may be a stable cycle; the cyclic solutions tend to arise when the carrying capacity of the fox habitat is relatively large (K significantly larger than K_T).

Figure 3 shows the types of dynamical behaviour which can occur, as functions of the latency interval $1/\sigma$ and the density of the disease-free fox population K , with the other epidemiological parameters having the values listed in Table 1b. The values of σ and K discussed above can be seen to suggest cycles with periods in the general range of 3–5 yr. Such periods are precisely what are observed in Europe and North America, where a striking feature of rabies epidemiology is the regular 3-, 4- or 5-yr oscillations in fox density and in the prevalence of infection^{6,14,18,24,27,66–69}. The cycles are most commonly observed in regions where fox densities were high before the introduction of rabies, and cyclic behaviour is often absent in low density populations¹⁵; these tendencies agree with the predictions of the model, as shown in Fig. 3. Qualitative insight into these properties of our model may be obtained by noting that the period of the cycle is determined primarily by the intrinsic growth rate, r , of the fox population (although, as Fig. 3 shows, other factors also influence the period), with rabies acting as a time-delayed density-dependent regulator of fox population growth (the length of the time lag being determined by how long

the fox density is below K_T); it is well understood that such mechanisms can induce oscillatory behaviour⁷⁰.

For the parameter values most relevant to the epidemiology of rabies in Europe ($1/\sigma \approx 30$ days, K in the region 1–4 foxes per km^2), Fig. 3 shows the model to predict damped oscillations in fox density and disease prevalence, with periods around 3–4 yr, leading eventually to a stable disease-controlled equilibrium. As illustrated in Fig. 4, however, the damping time is long, being of the order of 40 yr or more from the first appearance of the disease. This propensity to prolonged oscillations, even in the dynamical regime labelled 'damped oscillations' (Fig. 4), is further accentuated by seasonal changes in the values of pertinent parameters. Fox reproduction is clearly seasonal^{19,20}, and in addition the behavioural ecology of the fox (particularly mating and dispersal patterns) imposes a seasonal trend on disease transmission, which generates a marked peak in disease prevalence from January to April^{14,15,24,27}. Numerical studies show that seasonality in the transmission parameter β accentuates the non-seasonal oscillations in prevalence and fox density, considerably lengthening, for example, the damping time in the system portrayed in Fig. 4⁷¹. In short, the model gives a fairly robust prediction of 3–5-yr cycles in European fox populations infected with rabies¹⁰¹.

Before leaving the subject of cycles, one further complication deserves mention. In Northern Europe, 4-yr cycles have been reported for rabies-free fox populations; these cycles are likely to be a consequence of the well known 4-yr cycles in populations of microtine rodents in northerly regions^{27,28,72–75}. We are, however, not aware of any such cycles in rabies-free fox populations in Central or Southern Europe.

As a direct consequence of its high pathogenicity, rabies will only persist within fox populations at very low levels of prevalence; this is an example of the commonly observed biological relationship between low 'standing crop' and high rate of turnover. Persistence is in part ensured by the high susceptibility of foxes to rabies. In endemic areas of stable rabies, prevalence seems to lie in the range of 3–7% (ref. 76), although significant fluctuations are caused by seasonal effects and by the intrinsically oscillatory nature of the interaction between host and pathogen. As illustrated in Fig. 2b, our model gives prevalence values which agree with those observed. Moreover, the model predicts that the prevalence of rabies will be higher in favourable fox habitats (large K), which is consistent with the epidemiological evidence in Germany^{14,27,77}.

During the troughs in fox abundance (see Fig. 4) the prevalence may drop to very low levels. In these circumstances, the chance of stochastic effects causing extinction of the disease is high, particularly in small areas where the fox habitat is good (and consequently the amplitude of the cycles is large). The persistence of disease over large areas is probably ensured by the high spatial mobility of the fox host; young males may disperse

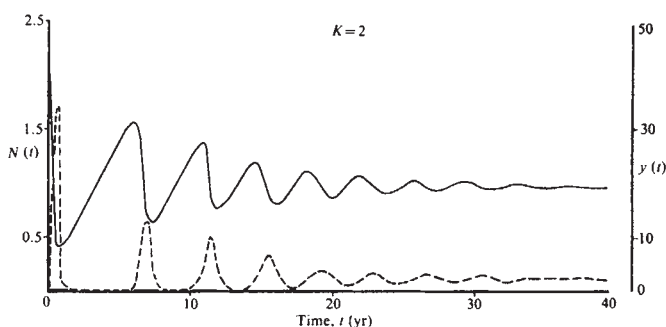


Fig. 4 A numerical solution of the model defined in equations (2) to (4) using the parameter values of Table 1b. The carrying capacity of the fox habitat, K , is set at 2 animals per km^2 and $K_T = 1$ fox per km^2 . The solid line represents fox density at time t , $N(t)$, and the dashed line the prevalence of infection, $y(t)$.

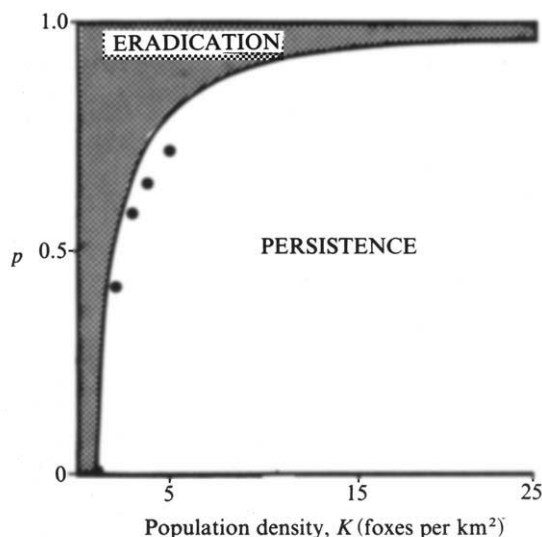


Fig. 5 This figure depicts the relationship between the proportion of the fox population p , protected by vaccination, and the disease-free carrying capacity of the fox habitat K ; the threshold density is taken to be $K_T = 1.0$ foxes per km^2 . In the shaded region equation (11) in the text is satisfied and rabies cannot persist; in the unshaded region equation (11) is not satisfied and the disease maintains itself. The observation of equation (11) is based on an extension of the model defined in equations (2) to (4) in the text, to include an immune category of foxes of density $Z(t)$ at time t ; susceptible animals are vaccinated at a *per capita* rate ϕ and immune animals lose immunity at a *per capita* rate δ . The new model is $dX/dt = a(X+Z) - (b+\gamma N)X - \beta XY - \phi X + \delta Z$, $dI/dt = \beta XY - (\sigma + b + \gamma N)I$, $dY/dt = \sigma I - (\alpha + b + \gamma N)Y$, $dZ/dt = \phi X - (\delta + b + \gamma N)Z$. For disease eradication the equilibrium solution (obtained by setting $dX/dt = dI/dt = dY/dt = dZ/dt = 0$) of interest is the disease-free state defined by $Y^* = I^* = 0$ and $N^* = K$. This state is locally stable provided $\phi > (a + \delta)(R - 1)$, and the proportion of vaccinated foxes, p , is $p = Z^*/(Z^* + X^*) = \phi/(\phi + a + \delta)$ which may alternatively be expressed as equation (11) in the text. The four solid circles are the numerical results obtained by Berger³⁴ from a very detailed model, as discussed more fully in the text. The circles denote the boundary, predicted by Berger's model, between levels of vaccination which result in either disease persistence or eradication.

13 km or more from their birth place, and adult foxes often travel 11–13 km each night^{19,20}. Empty territories created by deaths are rapidly occupied by itinerant and mobile male foxes²⁰. The involvement of other host species may also be important for the maintenance of endemic rabies during periods when fox abundance is low.

Control by reducing fox density

Attempts to control the spread of rabies in Europe and North America by hunting, gassing, poisoning or trapping foxes have generally met with little success^{8,15,19,24,30,76,78}; the disease is still endemic in Poland and West Germany despite intensive efforts to reduce fox density^{14,27}. Theory suggests the aim of such programmes should be to maintain fox numbers below the threshold density K_T , given by equation (7). Encouraged by the agreement with observed epidemiological patterns, we now apply our model to explore this problem.

There is a large body of literature, particularly for fisheries and whaling, dealing with the dynamics of natural populations under various regimes of culling or harvesting^{79–81}. One approach is to set a constant quota of animals, Λ , to be removed annually in order to keep the fox density at some specified level, below K . In the absence of rabies, the dynamics of the fox population under such culling obeys

$$dN/dt = rN(1 - N/K) - \Lambda \quad (8)$$

The analysis of this situation now follows standard lines laid down in the fisheries literature^{79–81}. Two cases arise.

If $K_T > \frac{1}{2}K$, the threshold density lies above the point where the fox population growth curve has its maximum (the 'maximum sustainable yield', MSY, point), and stable control to a density below K_T is possible, provided that the culling rate Λ exceeds Λ_T

$$\Lambda_T = rK_T(1 - K_T/K) \quad (9)$$

For K_T in the region of 1 fox per km^2 , this regime applies to areas of poor fox habitat, where K is below 2 foxes per km^2 ; culling can maintain the fox population at a stable equilibrium density $N^* < K_T$.

If $K_T < \frac{1}{2}K$, two complications arise. First, the initial quota must exceed the MSY value, $\Lambda > rK/4$, to drive fox densities below the point (at $\frac{1}{2}K$) where the population growth curve has its peak. Second, the fox population cannot be stably maintained at a finite value below $\frac{1}{2}K$ by culling; the equilibrium point at K_T , attained by culling at the rate Λ_T of equation (9), is unstable. In principle, the population could be driven to extinction by operating at $\Lambda > rK/4$, but in practice a prohibitively large effort would be required to meet this quota when fox density was very low. This regime applies to areas of good fox habitat where $K > 2$ foxes per km^2 ; such populations will be difficult to control by culling because of the continually high effort needed to keep an area free of foxes, given their ability to disperse over large areas and rapidly to recolonize vacant territories^{18,19,82,83}.

A different approach is required to devote some specified constant effort (for example, constant number of man-hours) to killing foxes. This regime, in effect, changes the intrinsic *per capita* death rate of foxes from b to $b + \Delta b$, where Δb is the additional mortality imposed by constant effort culling. Fox densities will be maintained below K_T , and rabies eradicated from the population, provided

$$\Delta b/r > 1 - 1/R \quad (10)$$

Here, R is the 'basic reproductive rate' for rabies in the original population, as defined in equation (6). Again, we see that if R is large, as it typically is in good fox habitat, Δb must essentially equal r itself, which implies a considerable level of effort. The practical implications of these predictions need to be assessed particularly with respect to the relationship between effort (determined by r and K), fox density and the rate of mortality achieved by control measures.

Other, more elaborate, culling regimes could be considered, but the broad theoretical conclusion remains that the control of rabies by culling foxes will be difficult to achieve in all except poor habitats, and even in these continual effort must be applied. Such conclusions agree with recent experience in Europe, and are a consequence of the high reproductive potential and dispersal behaviours of foxes^{18,20,82,83}.

The use of pathogens such as canine distemper or canine hepatitis as suppressors of fox numbers may be a feasible alternative, provided the threshold density for maintenance of the introduced pathogen is lower than that for rabies. Field observations in North America suggest that a more detailed appraisal of this approach is warranted⁶⁶.

Control by vaccination

The possibilities of immunizing wild foxes against rabies have been vigorously pursued in recent years^{84–87}, even though many problems surround this approach (not the least being the danger than an attenuated live vaccine which effectively immunized foxes might introduce a mild form of the disease into other host species)^{1,19,88}. Substantial progress has been made, however, in the development of oral vaccines and in techniques for delivering them in baits^{88–90}. Extensive field trials have been carried out, for example, in Canada, Germany and Switzerland^{19,89,91,92}. In the design of these trials, there has been much discussion about the level of protection needed within the fox population to eliminate rabies^{19,34,36,43}.

Our model provides an answer: the proportion of the population, p , that must be protected at any one time is simply

$$p > 1 - 1/R \quad (11)$$

Here, R is as defined in equation (6) (see also Fig. 5 legend)^{60,61,71}. This general relation (11) is illustrated in Fig. 5. Because R may equivalently be written as $R = K/K_T$, we see that K_T is the only parameter involved in specifying p as a function of K .

Figure 5 also displays the results of an elaborate numerical simulation by Berger³⁴. His model involves a detailed statistical description of dispersal by, and contact between, foxes on a large two-dimensional grid, along with statistical distributions for the duration of latent and infectious states in individual foxes. Like us, Berger took K_T to be 1 fox per km², and there is good agreement between the results of this detailed study and the simple equation (11); Fig. 5 provides a good example of the basic understanding that can be gained from simple models.

Equation (11) and Fig. 5 show clearly that the required degree of protection is greater in dense fox populations than in sparse ones; for K around 2 foxes per km², roughly 50% of the population must be protected (given the K_T of Table 1b). Vaccination thus seems to be a better method than culling, particularly in view of the 74% level of protection achieved by Johnston in Canada^{19,93} and the 60–70% bait acceptance attained in Germany⁸⁹. In good fox habitats, however, the required protection level approaches 100% (see Fig. 5), and will be difficult to achieve.

Control by vaccination and culling

If vaccination (of a proportion p of the fox population) is combined with constant effort culling (which adds Δb to the fox death rate), the criterion for eradication of rabies becomes

$$p + q - pq > 1 - 1/R \quad (12)$$

Here, $q = \Delta b/r$. This criterion generalizes equations (10) and (11), to provide an explicit estimate of the efficacy of a combined strategy. For instance, if $R = 4$ we would need to vaccinate 75% of the foxes, or to kill at a rate $\Delta b/r = 75\%$, under a pure strategy; a combined strategy would require, for example, $p = 50\%$ and $\Delta b/r = 50\%$. Without making any estimate of the relative costs, it does seem likely that the expenses of vaccination or of culling will rise faster than linearly with increasing p or Δb , so that the combined strategy may often be the most cost effective (as well as permitting more foxes to live than does pure culling).

Conclusion

In the event of an outbreak of rabies among wildlife in Britain, the government's official policy is to concentrate on the destruction of foxes^{18,19,94}. Our theoretical results, analysed in the light of available data, support MacDonald's conclusion¹⁹ that such an approach is unlikely to be effective once rabies becomes established within the fox population^{83,92,95}. Vaccination, conditional on the development of a 'safe' vaccine⁹⁶, and supported by culling in low density fox populations, is more likely to be successful. Our model provides a quantitative framework for discussing the precise level of control (whether vaccination, or culling, or a mixture) required in different fox habitats.

In view of the predicted difficulties of controlling rabies within areas of good fox habitat, the most sensible priority would be the compulsory vaccination of dogs and cats, along with eradication of strays and feral populations, so as to minimize the risk of human exposure in urban and suburban regions of Britain.

When assessing the significance of these conclusions it is clearly important to bear in mind the limitations of simple deterministic models (based on assumptions of homogeneous mixing), which ignore spatial and stochastic effects, and the accuracy of the data base used for parameter estimation. The power of simple formulations lies in the relative ease with which relationships can be established between important variables, a facility rarely provided for by complex simulation models (see equation (12) and Fig. 5). We emphasize, however, that our model predicts patterns of population behaviour in striking agreement with observed epidemiological trends. This leads us to believe that, by the specification of a few easily measured parameters, the model captures the significant features of the population association between host and pathogen and is thus of practical value to the design and implementation of control measures. To improve the accuracy of model predictions, however, additional data are ideally required, particularly with respect to the threshold density, K_T , the demography of fox populations in Britain and the dispersal behaviour plus contact rates of rabid foxes.

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The molecular structure and stability of the eye lens: X-ray analysis of γ -crystallin II

Tom Blundell, Peter Lindley, Linda Miller, David Moss, Christine Slingsby, Ian Tickle, Bill Turnell & Graeme Wistow

Laboratory of Molecular Biology, Department of Crystallography, Birkbeck College, University of London, London WC1E 7HX, UK

The three-dimensional structure of the eye lens protein, bovine γ -crystallin II, has been determined at 2.6 Å resolution. The protein has a two domain β -structure, folded into four remarkably similar 'Greek key' motifs, and shows the highest internal symmetry of any protein studied by X-ray analysis. Although the symmetrical structure seems very stable, the arrangement of the sulphhydryl groups would allow intramolecular cross-linking leading to possible destabilization, and intermolecular cross-linking leading to aggregation, both of which may be important in cataract formation.

ALTHOUGH eye lenses have evolved to suit the requirements of many ecological niches—from the very hard lens of the short-sighted rat to the very soft lens of birds capable of wide-ranging accommodation—all vertebrate lenses have a similar cellular structure comprising long fibre cells which pack hexagonally in spherical laminae. These grow throughout life, differentiating from the surface epithelial cells and overlaying older cells, so that the core of the adult lens is formed from the original fetal structure.

The transparency and refractive power of the normal lens depends on a smooth gradient of refractive index for visible light. This is achieved not only by the regular arrangement of the fibre cells, but also by a smoothly changing concentration gradient of lens-specific proteins, the crystallins, and other cell components.

The largest of the soluble crystallins, α -crystallin, is an oligomeric globular protein of molecular weight (MW) ~800,000. There are two gene-product subunits, α_2 and α_{B2} , both of MW^{1,2,3} 20,000. A proportion of these undergo post-translational modification⁴, which contributes to the considerable

heterogeneity and polymerization of native α -crystallin^{5,6}. β -Crystallins are also a heterogeneous class of proteins which exist as oligomers of several different sizes^{7,8}; the subunits vary in MW between 20,000 and 32,000 with the predominant species, β Bp, of MW 26,000 (ref. 9). γ -Crystallins, however, are monomers of 20,000 MW¹⁰, with highly homologous sequences^{11,12}. They show no similarity to α -crystallin, but a striking homology to the sequence of β Bp-crystallin has been recently observed¹³. γ -Crystallin II, the major species in the bovine lens, has six free sulphhydryl residues¹¹.

Little is known of the conformation and intermolecular interactions of the crystallins which give rise to the supra-molecular organization within the fibre cells. However, it is established that the increased light scattering of senile nuclear cataracts is associated with protein aggregation^{14–16}, together with chemical cross-linking, including disulphide bridges^{17–20} and denaturation²¹.

Here we report the three-dimensional structure of bovine γ -crystallin II at 2.6 Å resolution—the first X-ray analysis of a lens protein. The protein has a two-domain β -structure, folded

Table 1 Phasing statistics for the five heavy-atom derivatives given in resolution ranges

Reagent	Uranyl acetate	Thiomersal		Potassium auricyanide		Ethyl mercury chloride	Sodium hexachloroiridate (IV)	
Resolution	16→4.5 Å	16→3.4 Å	3.4→2.6 Å	16→3.4 Å	3.4→2.6 Å	16→3.4 Å	16→3.3 Å	3.3→2.8 Å
$\bar{F}_{PH}$	323.7	321.1	209.4	318.8	208.5	296.8	313.9	220.2
$\bar{F}_H$	35.7	64.9	44.3	51.7	41.9	100.1	54.4	40.2
E_H	40.9	37.2	35.0	39.5	37.4	66.6	60.1	43.0

$\bar{F}_{PH}$, mean value for the derivative protein structure factor amplitude. $\bar{F}_H$, mean value of the contribution of the heavy atom to the structure factor of the derivative. E_H , r.m.s. lack of closure²⁷ for the heavy-atom derivative.

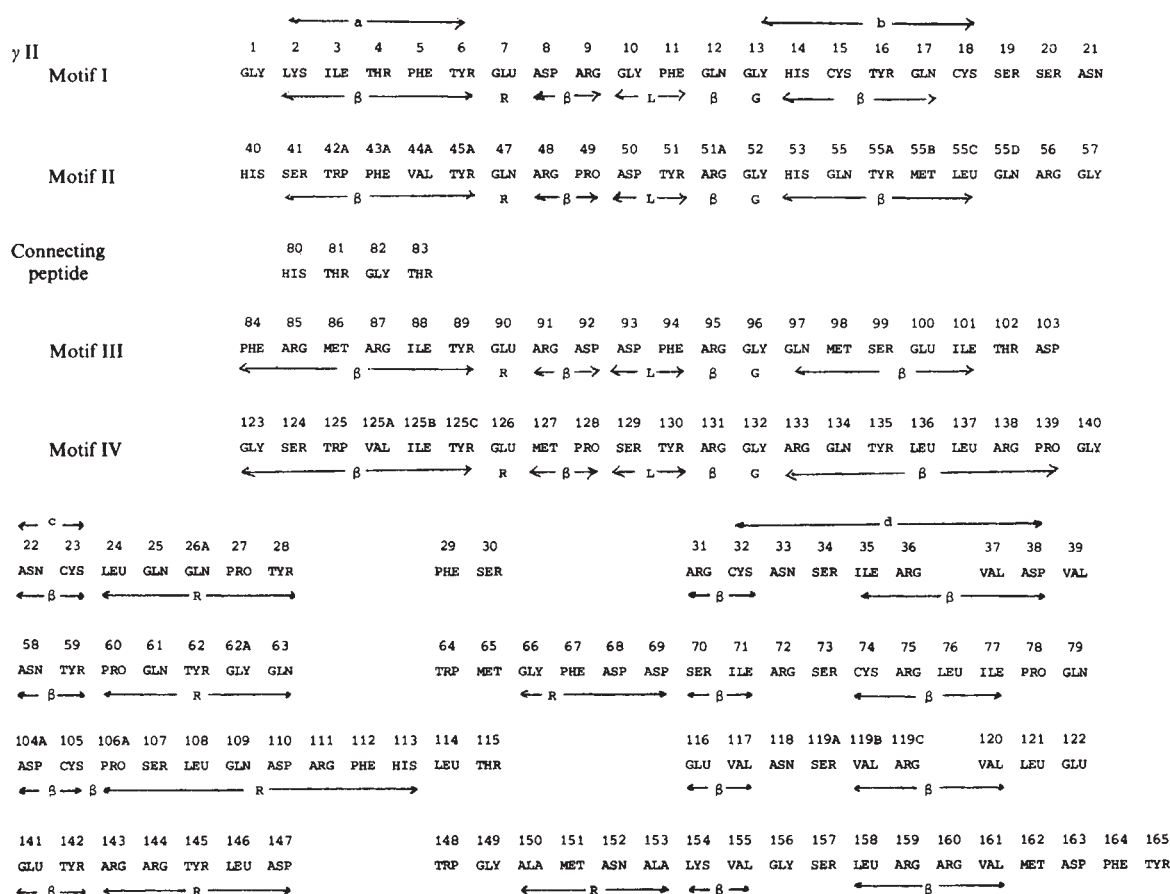


Fig. 1 The sequence of γ -crystallin II. The numbering is that of Croft¹¹ and subscripts indicate insertions or replacements necessitated by the electron density map. These generally improve the homology with the sequence of β Bp-crystallin reported by De Jong *et al.*¹³. The sequence is divided into four major, approximately equal parts which correspond to the four Greek key motifs, each with four strands: a, b, c and d. Topologically equivalent residues are in columns and the secondary structure indicated by the torsion angles is given: β , β -sheet; R, right-handed helix generally distorted from α -helix; L, left-handed helix, and G, a conformation found usually with glycine, with $\phi \sim 110^\circ$ and $\psi \sim -110^\circ$. The connecting peptide links the two domains.

into four remarkably similar Greek key motifs²²; it shows the highest internal symmetry of any protein studied by X-ray analysis. Each domain comprises a sandwich of two four-stranded sheets, each sheet being made from three strands of one 'Greek key' motif and one strand from another. The sequence shows a functional conservation of a few important residues in each of these motifs which had not been recognised earlier. This repeating structure, which may have evolved through gene multiplication, could be of general significance to other lens proteins in view of the sequence homology with β Bp-crystallin¹³. Although the symmetrical structure has the appearance of great stability, the sulphhydryl groups are arranged in ways which could allow both intramolecular and intermolecular cross-linking, and reaction with certain sulphhydryls could be expected to destabilize the conformation and lead to denaturation. The arrangement of charged and non-polar groups on the molecular surface has implications for the stability of the molecule, the nature of the hydration around lens proteins and the intermolecular contacts which may be important to the supramolecular organization of crystallins in the normal lens.

X-ray analysis

The γ -crystallins were isolated from calf lens using gel filtration on G-75 Sephadex to separate the α - and β -crystallins²³, ion-exchange chromatography on DEAE-Sephadex to separate the β s-crystallin (MW 28,000)²⁴, and ion-exchange chromatography on sulphopropyl-Sephadex and DEAE-cellulose to separate the γ -crystallins I, II, III and IV (refs 25, 26). Large crystals of γ -crystallin II were grown from 0.05M phosphate buffer, 1mM dithiothreitol, pH 7, at 0 °C (ref. 26). The crystals

were stabilized by cross-linking overnight in 3% glutaraldehyde in 0.05M sodium phosphate buffer, pH 7. Four heavy-atom derivatives were prepared using uranyl acetate, thiomersal, potassium auricyanide and ethyl mercury chloride as described elsewhere²⁷ and a further derivative using sodium hexachloroiridate (IV). Data were collected on a Hilger-Watts four-circle diffractometer with Ni-filtered $\text{CuK}\alpha$ radiation to the resolutions indicated in Table 1. Typically, ω scans with 1° scan width and 50-s counting time including background were analysed using a moving-window technique²⁸ to obtain the net integrated intensity. Two equivalents (hkl and $kh\bar{l}$) for the native and four equivalents (hkl , $kh\bar{l}$, $hk\bar{l}$, $kh\bar{l}$) for the derivatives were measured so that two estimates of the anomalous difference of each reflection for the derivatives were obtained. Empirical absorption corrections were applied using a modification of the method of North *et al.*²⁹ and radiation decay was monitored by standard reflections measured every hour. The heavy-atom positions were determined using F_{HLE} Patterson syntheses and refinement (see ref. 30 for review) and the relative hands and origins of the individual derivatives, and the absolute hand of the model, were established using cross-phase difference Fourier syntheses incorporating the anomalous differences²⁷. Phases were calculated using the method of Blow and Crick³¹ and the phasing statistics are summarized in Table 1. The mean figure of merit was 0.89 at 5.5 Å resolution but fell to 0.70 in the range 3.0–2.6 Å resolution.

A 'mini' electron density map at 3.4 Å resolution was calculated at a scale of 1 cm = 3 Å and the path of the polypeptide was followed. The 2.6 Å electron density map was initially interpreted using an optical comparator³² (scale 1.25 cm = 1 Å) and the α - and β -carbon, carbonyl oxygen and some side-chain

positions of this model were recorded. These were then displayed on an Evans and Sutherland Picture System II using FRODO³³ modified and extended by A. Jones and I. J. Tickle. The backbone atoms were generated using the input coordinates as guide points, with the model-building routine of Hermans³⁴, in the FRODO program and the coordinates improved interactively using computer graphics. The addition of side chains presented problems as some modifications to the sequence published by Croft¹¹ were required mainly in the hydrophobic regions. Our sequence (Fig. 1) is an attempt to fit the observed electron density but at the same time to minimize the number of implied errors in tryptic and cyanogen bromide peptide sequences which were used to derive the primary structure. The new sequence has higher homology to the sequence of β Bp (ref. 13) and also higher internal homology (*vide infra*) than that published previously¹¹. The numbering scheme uses alphabetical postscripts where the residues differ from the Croft sequence.

The topology of the folding units was compared using EZIFIT of A. D. McLachlan³⁵. Thirty-six topologically equivalent residues were compared, including all residues shown in Fig. 1, and equivalent to residues 1–37 with the exception of residue 21. This program optimizes the fit without assuming a strict 2-fold rotational symmetry. A program written independently by W.G.T. was used to find the agreement to a rotation of 180°. The motifs were also compared using interactive computer graphics to optimize their overlap, using a program FITZ written by Dr G. Taylor of our laboratory.

Symmetry and stability

The low-resolution electron density map²⁷ showed that the γ -crystallin molecule is ellipsoidal and organized into two well defined globular domains. It can now be seen that the N-terminal half of the sequence comprises one domain and the C-terminal half the second; the overall topology of the two domains is strikingly similar. Each domain is organized into two folding units (see Figs 2–4) that adopt a structure resembling a 'Greek key' motif²² shown schematically in Fig. 3b. We have divided the sequence into four sections of about 40 residues, labelled I–IV in Fig. 1, so that I and II correspond to the Greek key motifs in the N-terminal domain and III and IV correspond

to the motifs of the C-terminal domain. There is a short connecting peptide between the two domains, which are related by an approximate diad axis so that 80 α -carbon atoms of the N-terminal domain (38 of motif I and 42 of motif II) are topologically equivalent to those of the C-terminal domain with root mean square (r.m.s.) distance at best fit of 1.42 Å as calculated using EZIFIT³⁵ (rotation angle, 178.1°; screw shift, 0.14 Å).

We compared 36 common, topologically equivalent α -carbon atoms of each motif. For motifs I and II related by an approximate diad within the N-terminal domain, the r.m.s. distance was 2.40 Å (rotation angle, 179.3°; screw shift, 1.32 Å). Motifs III and IV in the C-terminal domain similarly gave a r.m.s. distance of 2.76 Å (rotation angle, 179.8°; screw shift, 1.12 Å). However, the most striking relationship is between motifs II and IV, where 42 consecutive α -carbon atoms in the two motifs are related with a r.m.s. distance of 1.05 Å, and between motifs I and III where 36 topologically equivalent residues are related with a r.m.s. distance of 1.74 Å.

The diad relating atoms of the two domains bisects the angle of $\sim 40^\circ$ between the diads relating folding units, all three diads being approximately coplanar. These observations demonstrate that folding units I and III are similar, as are II and IV. There is a less close correspondence between the folding units within one domain—this is illustrated in Fig. 2 which compares the α -carbons of the folding units.

Given the similarities between the Greek key motifs it is convenient to first describe motif I and then to relate the others to this. The sequence in Fig. 1 has been arranged so that topologically equivalent residues in each motif occur in one column, and an indication of the conformation of residues is included so that secondary structures can be inferred.

Each 'Greek key' comprises four antiparallel strands. In motif I (Fig. 3a), residues 2–6 (strand a) form an extended chain in β -sheet conformation that runs antiparallel and is hydrogen-bonded to residues 14–18 (strand b). The turn between residues 7 and 13 is made possible by Glu 7 assuming a right-handed helical conformation and Gly 13 having the ϕ, ψ values of 126° and -116° , respectively, which are normally disallowed for residues with side chains. Residues 10 and 11 assume the conformation of a left-handed α -helix so that the side chain of

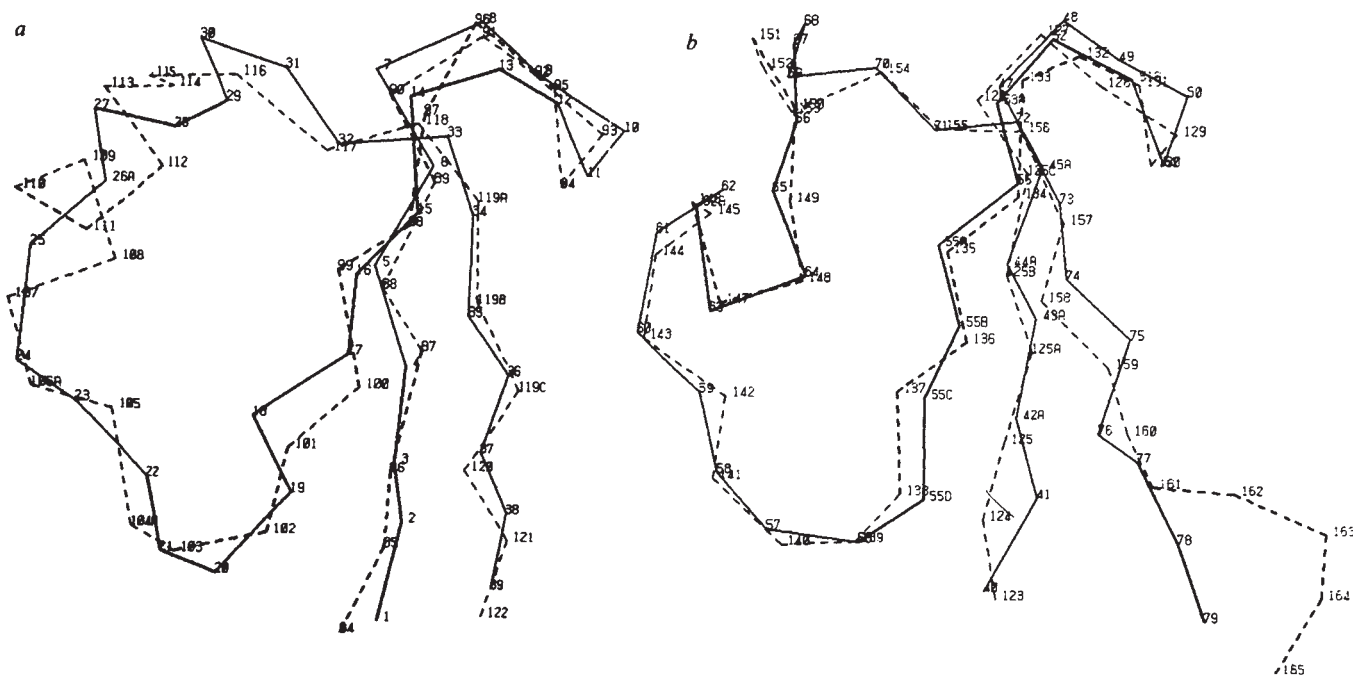


Fig. 2 The path of the polypeptide chain is illustrated by the positions of the α -carbon atoms for each of the folding motifs defined in Fig. 1. *a* Shows the best fit of motifs I and III obtained visually using interactive computer graphics (program FITZ of Dr G. Taylor). *b*, A similar best fit from an equivalent direction for motifs II and IV, which show the closest resemblance in sequences between the topologically equivalent residues.

Phe 11 is packed on to the antiparallel sheet against Tyr 6. The structure of residues 1–18 may therefore be described as a two-stranded antiparallel β -pleated sheet which has a turn at residues 10 and 11 and which is folded at residues 7 and 13.

Residues 19–21 form a further turn so that residues 22 and 23 lie antiparallel and adjacent to residues of strand b. After residues 22 and 23, which have a β -sheet conformation, the chain assumes a rather irregular, right-handed helical structure between residues 24 and 28. The following residues form a gentle bend so that residues 32–38 form strand d, which is hydrogen-bonded as part of the antiparallel β -sheet including strands a and b.

Figures 2–4 indicate that the conformations of motifs II, III and IV may be derived from that of motif I by insertions and small changes in conformation. The major differences between motifs I and III are the deletion of one residue in the turn between strands b and c and the insertion of three residues as a continuation of the right-handed helical region at the end of strand c. Motifs II and IV are slightly larger than I, with four insertions in the loop between the helical region at the end of strand c and strand d. Figure 4 shows that the hydrogen bonding in the β -sheets is similar.

The Greek key motifs pack together so that each domain comprises two right-handed, twisted, antiparallel β -sheets sandwiched together (Fig. 3c). The fourth strand of each sheet is comprised of strand c of the other motif in the same domain as

indicated in Fig. 4. As strand c is of a less regular β -sheet structure than the other strands, it makes few well defined hydrogen bonds. The angle between the planes of the β -sheets is $\sim 35^\circ$ giving the domains the wedge shape seen in Fig. 3c, and the angle between individual strands in opposing sheets projected on to the mean plane is $\sim 20^\circ$. The volume between the β -sheets is occupied by hydrophobic side chains; these are more predominantly aromatic near the top of the wedge. The top of the wedge-shaped domain is enclosed by the two antiparallel strands c and d of the two Greek key motifs. In motif I the following residues contribute to the core: 3, 5, 16, 18, 23, 28, 32, 35, 37; the topologically equivalent residues in Fig. 1 (columns beneath the residues listed) also contribute to the cores of the domains, making them compact and largely hydrophobic. Residues in strands b and c have their hydrophilic functional groups folded in such a way as to minimize the contact of the core with the solvent.

The two domains are packed together (Fig. 5) so that the two sheets involving strands a, b and d of motifs II and IV are sandwiched with an angle of 20° between the individual strands. The contacts are again largely hydrophobic and include Phe 43A and Met 55B of the N-terminal domain, and Val 125A and Leu 136 of the C-terminal lobe. The connecting peptide is accommodated so that it follows the surface of the molecule.

The structure of γ -crystallin shows the highest degree of intrachain symmetry of any protein whose three-dimensional structure has been elucidated. The eye lens proteins must exist throughout the life of the animal without denaturation, due to the fact that to avoid sharp changes in refractive index which would result from irregular structures, the fibre cells become denucleated and have few cellular organelles such as mitochondria and ribosomes. As there is little or no capacity for regeneration, the proteins must be relatively stable, and we suggest that the high degree of symmetry of these proteins may contribute towards this stability.

Gene multiplication in evolution?

The similarity in the three-dimensional structure of the motifs poses the question as to whether the structure may have evolved divergently in two stages: first, gene duplication and fusion of a protein of ~ 40 amino acid residues to a protein approximating the present γ -crystallin domain, followed by a further gene duplication to give the two-domain structure. This model is consistent with the observation that the two domains resemble each other more closely than do the two motifs within each domain. This is also evident from the sequence homologies

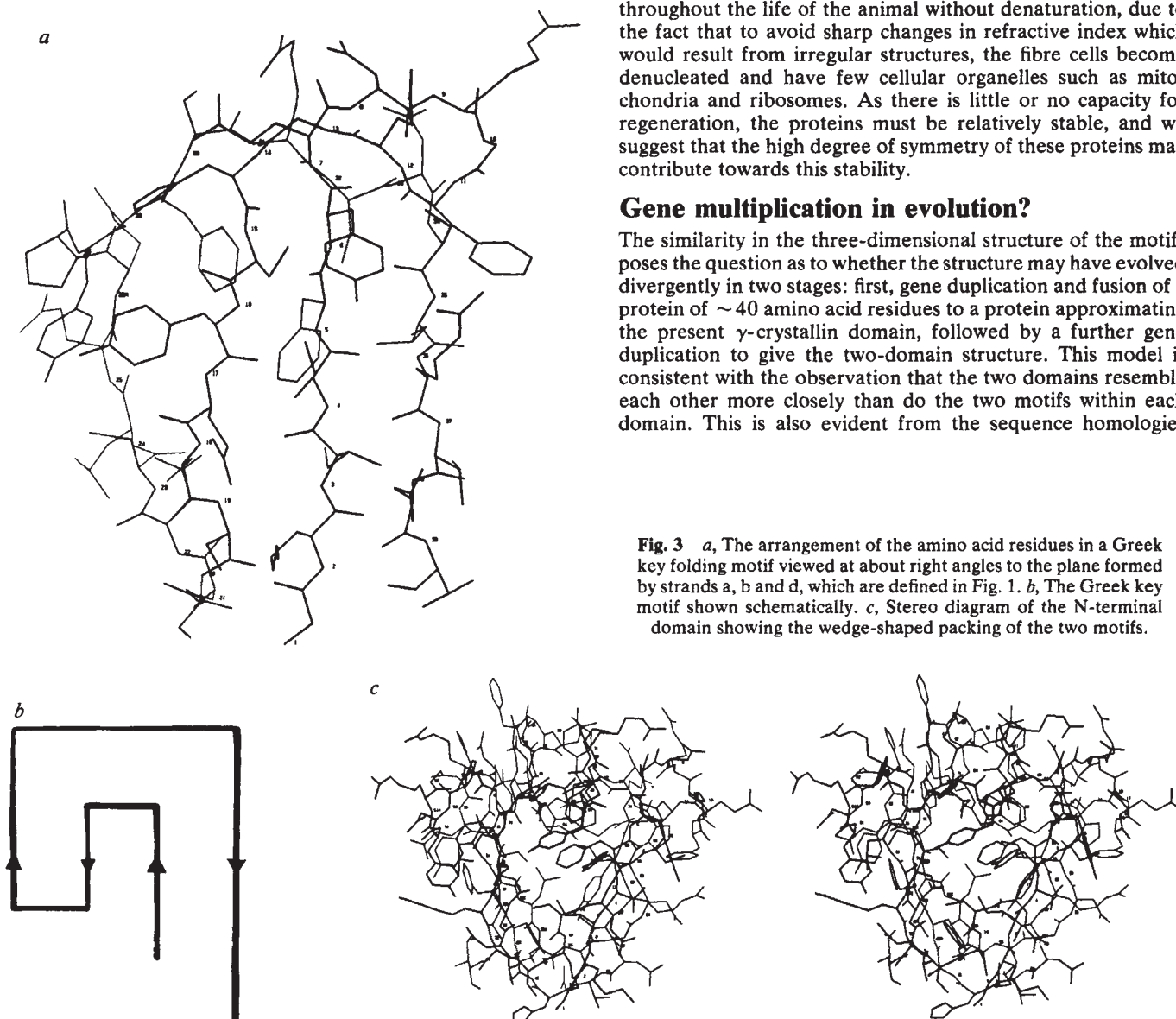


Fig. 3 a, The arrangement of the amino acid residues in a Greek key folding motif viewed at about right angles to the plane formed by strands a, b and d, which are defined in Fig. 1. b, The Greek key motif shown schematically. c, Stereo diagram of the N-terminal domain showing the wedge-shaped packing of the two motifs.

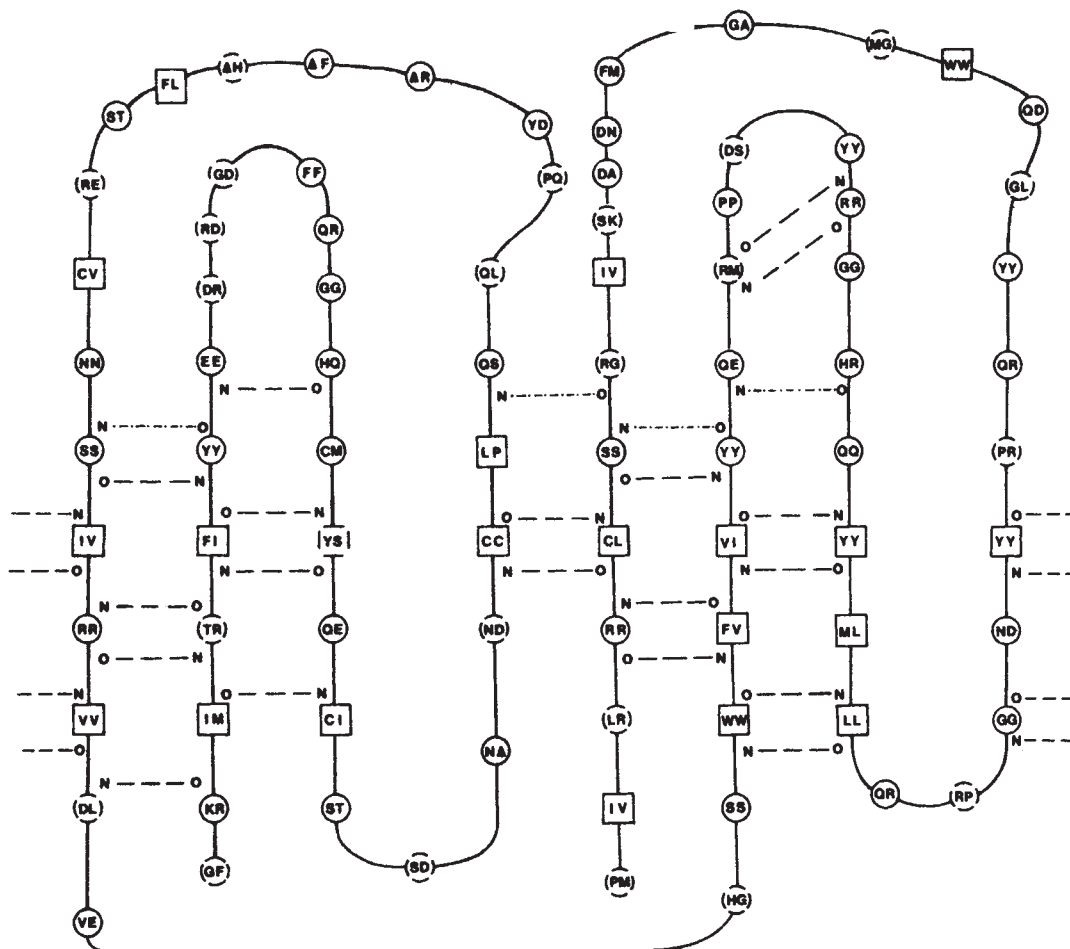


Fig. 4 The arrangement of the amino acids in a γ -crystallin domain shown schematically. Each box indicates the nature of the two topologically equivalent amino acids of the two domains. Deletions in one domain are given by Δ . Square boxes indicate residues which are directed either between the sheets within the domains or between the sheets between the domains; some of these residues in strands b and c (see Fig. 1 for definition) are on the edge of the sheets and are therefore partially accessible to solvent. The extent of the sheets is indicated by the length of strands shown in Fig. 1 and also by the hydrogen-bonding network. Those hydrogen bonds common between the two domains are indicated by ----- whereas those occurring in one domain only are indicated by

illustrated in Figs 1 and 4, which must, however, be read with caution as they involve inferences from the electron density map. The number of residues conserved at topologically equivalent positions between motifs is much greater for motifs II and IV than for the two motifs within either domain. These observations are consistent with the model for two sequential tandem duplications.

Only four residues are invariant at topologically equivalent positions between all folding units. These include Tyr 6 which has a structural role in packing against the conservatively varied Phe 11 (Tyr) of the folded β -turn between strands a and b (Fig. 6) and Gly 13 which has angles disallowed for other residues and allows the fold to occur. Glu 7, which is necessary for the folding

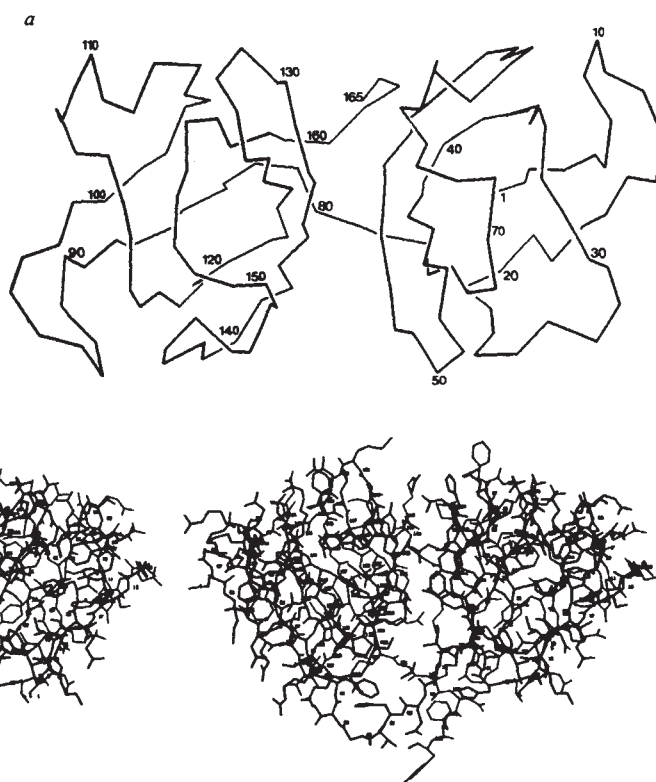


Fig. 5 The arrangement of the two domains in the molecule of γ -crystallin II. *a*, The C^α backbone viewed almost parallel to the pseudo 2-fold axis between the domains which are clearly shown. *b*, A stereo diagram of the complete molecule viewed approximately perpendicular to the plane, containing the three pseudo 2-fold axes.

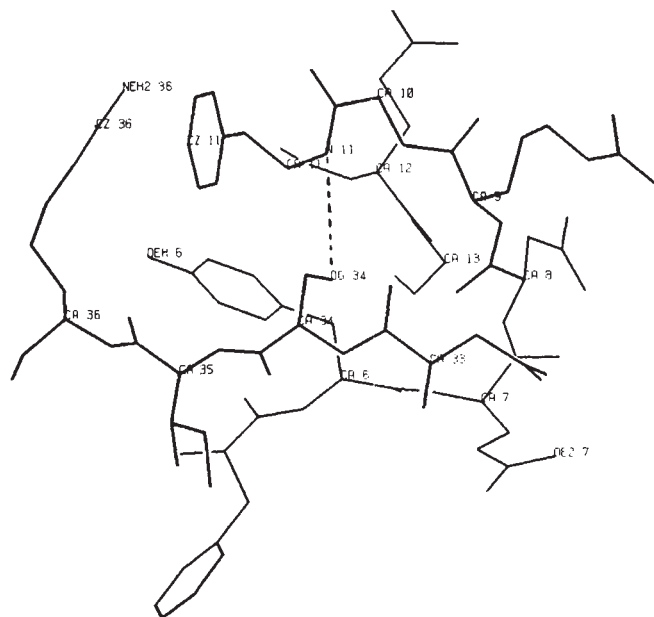


Fig. 6 The portion of motif I in the N-terminal domain containing residues invariant at topologically equivalent positions in the four motifs.

over of the β -structure, is very conservatively varied to Gln (in motif III alone). The third invariant residue, Ser 34, is also involved in this structure (see Fig. 6). The γ -O of this serine in each folding unit is relatively shielded from solvent and hydrogen bonds to the peptide nitrogen of the residue topologically equivalent to Phe 11 on the turn. This most unusual structural motif is further stabilized by the fourth invariant residue Arg 36, also shown in Fig. 6; this arginine seems to be well ordered and to have its aliphatic side chain against the aromatic ring of Phe 11 with its guanidinium group exposed to the solvent. The fact that this arrangement is strongly conserved both in three dimensions and in sequence underlines the importance of these amino acids, but their structural role would be consistent with convergent evolution as well as gene multiplication and divergent evolution.

One of the surprising aspects of the structure is that very few residues of the hydrophobic core are invariant. It seems that there are several ways in which the same volume occupation of side chains of the core can be achieved while still allowing a very close three-dimensional homology. Evolution—whether convergent or divergent—seems to have exploited these possibilities and it is this variation, combined with the insertions and deletions in the bends, which makes recognition of structural homology very difficult from sequence alone. If this is divergent evolution, here is further evidence that three-dimensional structure is more strongly conserved than sequence.

Surface characteristics

The surface of the γ -crystallin II molecule is characterized by a number of extremely well defined charged and polar side chains, many of which seem to be organized in pairs or chains of ionic and/or polar interactions. For example, Glu 7 and Asp 8 at the beginning of the fold between strands a and b of motif I have their acidic side chains lying on either side of the basic guanidinium moiety of Arg 31 at the beginning of strand d; Glu 116 is cracketed by Asn 118 and Asn 152; Glu 122, Arg 85, Glu 100 and Arg 87 form a chain of ionic interactions.

The most extensive network of charged groups is centred on Asp 92 and includes a series of six alternately positively and negatively charged side chains in one molecule (Fig. 7). However, one end of the chain, Arg 144, is close to Asp 103 of a symmetry-related molecule at a crystal lattice contact point so that the ionic interactions connect neighbouring molecules. In fact, Glu 90 and Arg 91, adjacent to Asp 92 but probably not

linked ionically, form an ion pair dimer with their symmetry-related equivalents Arg 91 and Glu 90 across a 2-fold axis to a third molecule in the lattice.

It seems likely that at the intramolecular level, these interactions are partially responsible for the high stability that seems to be functional in this structure. It has been pointed out³⁶ that enzymes from thermophilic bacteria have abundant surface ion pairs which contribute to thermal stability. An 'exoskeleton' of organized charged groups could therefore be quite important in holding together the strands that form each domain of γ -crystallin II.

On the level of intermolecular contacts, not only hydrophilic residues are involved. The few relatively exposed hydrophobic side chains, such as Phe 11 and Tyr 51 are often found at lattice contact points (Phe 11 at a 2-fold near its symmetry-related equivalent). Similar interactions may occur in the normal lens and contribute to its supramolecular organization and control of protein concentrations. Differences in the balance of these polar and non-polar surface residues may be responsible for the different properties of the various γ -crystallins, for example, their relative abilities to cryoprecipitate. A comparative study of γ -crystallins is now being done in our laboratory to investigate these effects (parallel work is being done by Dr Y. Chirgadze^{37,38}).

The nature of the molecular surface may also be important when considering the structure of the water in the lens. Eye lenses generally have a very low water content, particularly in the most central regions where, in mammals, γ -crystallins are most concentrated. The percentage of water correlates with the refractive power of the lens and its degree of softness and therefore its ability to accommodate. The charge interactions that fix the positions of polar side chains, making them so well defined in the electron density map, would lead to partial neutralization of charge, but by being fixed, the surface ions could still have a major effect in ordering local water. One of the roles of the crystallins may be to form hydration spheres and regulate the water content of the lens.

Sulphydryl arrangement and accessibility

It is surprising that a protein which must retain its conformation and seems to have a largely structural role should have evolved with six free sulphydryl groups. An intracellular protein of this size would be expected to have about two cysteine residues³⁹. It was therefore interesting to examine the accessibility, reactivity and arrangement of these groups.

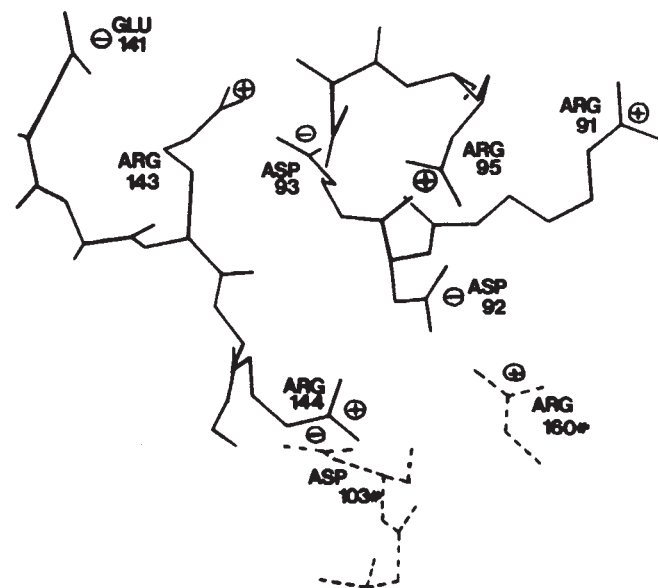


Fig. 7 An extensive network of charged groups on the surface of the molecule, centred on Asp 92. This and similar interactions may be partially responsible for the stability of the γ -crystallin molecule.

The three-dimensional structure demonstrates that not only do five of these groups lie in the same domain but three are also clustered closely together. Figure 4 indicates that Cys 23 and Cys 74 lie together on the same side of adjacent strands of a β -sheet in a position to form a disulphide bridge. Cys 18 also lies close by on the other sheet in the same domain, and this might also form an intramolecular disulphide bridge with Cys 23 in non-reducing conditions. Such intramolecular disulphide bridges would not radically affect the overall conformation of the molecule. Cys 15 and Cys 105, like Cys 23 and Cys 18, are on either strand b or c of the Greek key motif, and are therefore accessible to solvent. In the appropriate oxidizing conditions, these cysteines could form intermolecular disulphide bonds. In species such as the rat, where the core lens proteins undergo almost complete oxidation of protein sulphhydryl^{40,41}, a combination of inter- and intramolecular disulphide cross-links must occur, involving such surface and clustered cysteines.

The two remaining cysteine residues, 32 and 74, are relatively buried in the structure. We observed early on in our study that extensive reaction in the crystals with small, reactive groups such as ionic mercury salts led to a rapid loss of isomorphism, and that reaction in solution led to precipitation. This did not occur if the mercury was coordinated with softer anions, attached to large groups (as in thiomersal) or part of a small hydrophobic cation like EtHg^+ . The buried cysteine residues are not accessible to the large thiomersal group, whereas the small EtHg^+ can bind to

both, causing only small changes in conformation, as the ethyl group does not greatly disturb the hydrophobic core of the molecule. It is probable that Hg^{2+} ions bind to Cys 32 and 74 but then, by production of CysHg^+ , cause destabilization and possibly denaturation of the molecule. Such an irreversible covalent change leading to denaturation could be the first stage in a series of catastrophic and cascading events leading to the extensive polymerization and denaturation observed in senile nuclear cataracts. Whether these observations are relevant to human senile cataract must, however, await the chemical characterization of human γ -crystallin (Augusteyn *et al.*, unpublished results).

We have thus shown that although γ -crystallin II has a remarkably symmetrical structure which may confer stability on this protein in the normal healthy lens, the unusual arrangement of sulphhydryls could contribute to the process of cataract if the reducing environment of the lens, including glutathione levels, were disturbed. Furthermore, the existence of a highly organized arrangement of charges on the molecular surface may be important in the organization of the crystallins and water in the normal transparent lens.

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LETTERS

Constraints on heavy neutrinos

Doug Toussaint & Frank Wilczek

Institute for Theoretical Physics, University of California, Santa Barbara, California 93106, USA

If a heavy neutrino of mass $1.1 \text{ MeV} < m < 14 \text{ MeV}$ participates in the weak charged current coupling for the electron, then it can be produced in the Sun and decay in flight into an electron, a positron and a light neutrino. It is shown here that existing experimental bounds on low-energy interplanetary positrons severely constrain the heavy neutrino-electron coupling. Accelerator experiments severely constrain the coupling of still heavier neutrinos.

Several recent developments in high-energy physics and in astrophysics have reawakened interest in the question of neutrino masses. There have been experiments on the end-point energy in tritium decay¹ and on neutrino oscillations² which purport to show positive results. The tritium experiment is easier

to quantify, and if correct shows that at least one of the neutrinos emitted in tritium decay with large amplitude has a mass of a few tens of electron volts. Also, some unified theories of strong, electromagnetic and weak interactions suggest that neutrinos are massive³. In these theories leptons are treated in parallel with quarks, except that the right-handed components of the neutrinos are $\text{SU}(3) \times \text{SU}(2) \times \text{U}(1)$ singlets (having neither strong, electromagnetic nor weak interactions) and so can acquire a large Majorana mass. In these theories we often find mass formulae of the form

$$m_\nu \propto m_q^2/m_N \quad (1)$$

where m_ν is the neutrino mass, m_q is the mass of the corresponding charge $2/3$ quark (for example, u quark for the lightest neutrino, c quark for the next lightest) and m_N is the Majorana mass. The quark-lepton analogy might also suggest small mixing angles among different neutrinos, analogous to the Cabibbo angle for quarks. Finally, observational evidence for non-luminous matter has become quite strong⁴, reviving interest in suggestions that massive neutrinos could contribute significantly to the overall mass density in the Universe⁵ and in

galaxies and their haloes^{5,6}. In these regards it is neutrinos with mass of a few tens of electron volts, and lifetimes longer than the age of the Universe, that are most significant. Heavier stable or quasi-stable neutrinos are ruled out by these astrophysical considerations. (However, if the neutrino mass gets above several GeV, these limits no longer apply, as noted by Lee and Weinberg⁷.)

These considerations suggest that the lightest neutrino, coupling mainly to the electron, may have a mass of a few tens of electron volts. According to equation (1) we would expect the other neutrinos to be much heavier, and presumably unstable on cosmological time scales due to some coupling with the electron charged current through mixing. We will argue that existing observational evidence on the abundance of low-energy interplanetary positrons severely constrains the allowable mixing angles for neutrinos with mass $1.1 \text{ MeV} < m < 14 \text{ MeV}$. (Astrophysical constraints on very long-lived neutrinos which decay radiatively have been given by Cowsik⁸.) Accelerator experiments restrict the mixing angles for still heavier neutrinos, and could lead to very stringent limits.

Our astrophysical limits are based on consideration of heavy neutrinos produced in the Sun and decaying in interplanetary space. Neutrinos of mass up to 14 MeV can be produced in the Sun through the decay ${}^8\text{B} \rightarrow {}^8\text{Be} + e^+ + \nu$ in a side branch of the proton-proton chain. (These neutrinos are those searched for by R. Davis. He finds a factor three less than the lower theoretical estimate. We use the lower theoretical value here but appropriate factors of three may be applied.) If the coupling of the heavy neutrinos is a factor $\sin \theta_e$ of the total, then the expected heavy neutrino flux at Earth will be $F = \Phi \sin^2 \theta_e g(m)$ where $2.4 \times 10^6 < \Phi < 4.5 \times 10^6 \text{ cm}^{-2} \text{ s}^{-1}$ is the flux calculated for massless neutrinos⁹ and $g(m)$ is a factor taking into account the reduced phase space for ${}^8\text{B}$ decay ($g(0) = 1$). The decay rate Γ for heavy neutrinos into electron, positron and light neutrino is obtained by scaling from the μ lifetime as

$$\Gamma = \Gamma_{\mu \rightarrow e \nu \nu} (m_\nu / m_\mu)^5 \sin^2 \theta_e h(m_e / m_\nu) \\ = 5 \times 10^{-5} (m_\nu [\text{MeV}])^5 \sin^2 \theta_e h(m_e / m_\nu) \text{ s}^{-1} \quad (2)$$

where h is a phase-space factor with $h(0) = 1$. In the interesting range of parameters the lifetime of the heavy neutrino is so long that most of them decay outside the Earth's orbit. To calculate the flux of positrons at the Earth's orbit we simply multiply the neutrino flux by the probability of decay inside the Earth's orbit (actually this must be done energy by energy to take account of time dilatation).

Now we can compare these calculations with the relevant experiments¹⁰⁻¹². According to the latter, the flux of positrons in interplanetary space at around 5 MeV kinetic energy is $\approx 10^{-4} \text{ cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ MeV}^{-1}$. This flux is consistent with estimates of positron flux from cosmic-ray secondaries, so we take it as an upper limit on the possible flux from solar neutrino decay. Comparing with our calculations, we find severe constraints on the mixing angle. For example, at $m_\nu = 2 \text{ MeV}$ $\sin^2 \theta_e < 8 \times 10^{-5}$, at $m_\nu = 5 \text{ MeV}$ $\sin^2 \theta_e < 5 \times 10^{-6}$, at $m_\nu = 10 \text{ MeV}$ $\sin^2 \theta_e < 1 \times 10^{-6}$. The constraints continue to be quite severe until the mass gets very close to the kinematic limits—1.1 MeV for the e^+e^- decay and 14 MeV for production.

Neutrinos of mass $< 1.44 \text{ MeV}$ would also be produced in the reaction $p + p + e^- \rightarrow d + \nu$. The estimated neutrino flux from this reaction is $1.6 \times 10^8 \text{ cm}^{-2} \text{ s}^{-1}$, about two orders of magnitude larger than the flux from ${}^8\text{B} \rightarrow {}^8\text{Be}$, and because these neutrinos would have low energy, a larger fraction of them would decay inside the Earth's orbit. However, the experimental limit on positrons in this energy range is about two orders of magnitude higher than at 5 MeV, so the limit on $\sin^2 \theta_e$ is only slightly better.

Dicus *et al.*¹³ have derived constraints based on cosmological considerations which are complementary to ours. Heavy neutrinos which are long-lived are dangerous in cosmology: their decay can inject additional entropy into the Universe after nucleosynthesis, thus invalidating calculations of deuterium

abundance; or their decay can introduce non-thermal background radiation contrary to observation; or their failure to decay can cause contraction of the Universe as we mentioned before. The limits of Dicus *et al.* seem to overlap ours, so that if we take both the big-bang cosmology and solar neutrino production seriously, neutrinos in the mass range 1.1–14 MeV seem to be ruled out altogether. We leave aside the bizarre possibility of a heavy neutrino which although above the threshold for e^+e^- decays only into three neutrino channels.

Accelerator experiments can also put constraints on heavy neutrinos, using the same principles (with the Sun replaced by an accelerator). To get an idea of the magnitudes, let us compare the mean free path l_m for weak interactions of a neutrino with kinetic energy E with the decay length l_d for decay into e^+e^- .

$$l_m \approx (G^2 m_p E n)^{-1} = \frac{4 \times 10^{13}}{E [\text{GeV}]} \text{ cm} \\ l_d \approx 6 \times 10^{17} \frac{E [\text{GeV}]}{(m [\text{MeV}])^6 \sin^2 \theta_e} \text{ cm} \quad (3)$$

Therefore, neutrinos with masses of 50 MeV or so might be more likely to decay yielding an energetic e^+e^- pair than to undergo normal weak interactions in a large detector. To make the comparison fair, note that other mixing factors appear in the relative production rates for heavy versus light neutrinos. If the neutrino beam is generated from π decays, a heavy neutrino with mass $< 35 \text{ MeV}$ can come from $\pi^+ \rightarrow \mu^+ \nu$ with a mixing factor $\sin^2 \theta_\mu$ measuring its participation in the muon charged current. Another source could be $\pi^+ \rightarrow e^+ \nu$, with the usual kinematic suppression undone for heavy neutrinos: this would occur with probability $\sin^2 \theta_e (m_\nu / m_\mu)^2$ of the ordinary π decay. Similar considerations apply to K-decay which also has larger available phase space and could therefore produce heavier neutrinos.

Note that heavy neutrinos produced in $\pi^+ \rightarrow e^+ \nu$ decay would be largely helicity-flipped. If the neutrino mass is of Majorana type, then these helicity-flipped neutrinos would interact and decay as antineutrinos. The angular distribution of the e^+e^- pairs in the decay would be modified accordingly.

We have not examined carefully what quantitative conclusions can be drawn from existing experiments, but clearly heavy neutrinos with mass $> 50 \text{ MeV}$ would have to appear with small mixing angles. It would seem that very sensitive experiments could be designed to look for ν decays in flight, in particular at a K-meson factory.

The effect of such a massive neutrino on the e/μ ratio in K_{l2} and π_{l2} decays has been calculated by Goldman and Kolb¹⁴. Recently Shrock¹⁵ has proposed searching for unstable neutrinos or for characteristic energy shifts of the charged leptons in π_{l2} and K_{l2} decays, and Calaprice (S. Treiman, personal communication) has analysed deviations from universality in nuclear decays as possible signatures for large neutrino masses. These methods, of course, could in principle be sensitive to masses below 1.1 MeV.

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IR observations of the solar corona—a ring around the Sun?

U. R. Rao, T. K. Alex, V. S. Iyengar,
K. Kasturirangan, T. M. K. Marar, R. S. Mathur
& D. P. Sharma

ISRO Satellite Centre, Bangalore 560 058, India

The possibility of a permanent ring structure around the Sun was first pointed out by Brecher *et al.*¹ from evolutionary, physical and chemical considerations as well as constraints arising from observational astronomy related to Sun and its immediate neighbourhood. In particular, Brecher *et al.*¹ concluded that such a ring, located at $\sim 4 R_{\odot}$, must consist of refractory particles (graphite, for example) of size >10 km at $\sim 1,000$ – $2,000$ K which, therefore, would emit thermal radiation peaking around 1.5 – 3.0 μm . We report here our attempt to detect the existence of this ring structure utilizing the IR observations taken during optimal viewing conditions of the total solar eclipse of 16 February 1980.

The experimental system consisted of a 6-inch $f/6$ newtonian telescope coupled to a celostat. The detector assembly consisted of a PbS detector, a chopper with its motor, a filter and a detector cooling box. The 1×1 mm PbS detector, cooled to liquid nitrogen temperatures had an NEP of 1.3×10^{-10} W at a chopping frequency of 1.042 kHz. The interior of the cooling box was continuously purged by dry argon gas to prevent frosting of the filter-detector assembly. The IR filter with antireflection coating had a transmission of $\sim 75\%$ in the 1.9 – 2.8 μm range. The electronics consisted of a preamplifier (ORTEC model 5001), lock-in amplifier (ORTEC model 9501), chopper

reference circuit and a tape recorder. The entire detector assembly was mounted on a moving carriage that enabled bi-directional scanning of the corona in the image plane, up to $5.5 R_{\odot}$ on either side of the solar disk. The instrument was calibrated using the Moon at its various phases, and a black-body source.

The observations were made from Hosur (long $75^{\circ}09'04''$; lat $15^{\circ}00'12''$) in Karnataka, India where the duration of the eclipse totality was ~ 2.5 min. During totality five scans in the image plane were carried out along 9.5° , 5.5° , 1.5° , -2.5° and -6.5° with respect to the solar equatorial plane. The spatial resolution of the instrument was $0.25 R_{\odot}$. A continuous scan was made in the image plane.

Figure 1 shows the measured IR flux plotted as a function of distance from the centre of the Sun to a distance of $5.5 R_{\odot}$, corresponding to the scan mode close to the equatorial plane. The absolute brightness of the outer corona has been deduced using calibration data on the Moon at its various phases. Comparison of the flux obtained in the present experiment at $3 R_{\odot}$ with values obtained by Peterson² and MacQueen³ indicate that the present value is larger by ~ 3.5 and 6 times their values respectively. These differences can be reconciled, as the present observations coincided with the period of solar maximum and the earlier observations of Peterson² and MacQueen³ were made during the total solar eclipse of November 1966 at a time of solar minimum. Three independent observations⁴ also indicate the variabilities in the IR emission from the solar corona. The first observation, using a coronagraph during a 1971 balloon flight, apparently revealed a weak and diffuse emission around $4.3 R_{\odot}$ at 2.2 μm . The second observation, during a 1973 total solar eclipse revealed no evidence of a feature at $4 R_{\odot}$ nor of a shelf of emission out to $6 R_{\odot}$. In the third observation, a balloon flight after the 1973 eclipse also showed a weak feature, this time at $5 R_{\odot}$ elongation, but no shelf. Our observations also imply that the IR corona is highly variable. Continuous monitoring of the solar corona in the near IR is, therefore, important for understanding interplanetary dust models.

At $4 R_{\odot}$, the expected location of the ring, our measured flux is a factor of only 1.6 larger than the value obtained by Peterson². We did not, however, observe the excess flux above the continuum reported by Peterson² and MacQueen³. The present observations therefore do not support the existence of a ring in the equatorial plane of the Sun, corresponding to a 3σ sensitivity of 10^{-7} W cm^{-2} sr^{-1} μm^{-1} at 2.2 μm in our detection system. Other scans which were conducted away from the equatorial plane have shown similar results, namely the absence of IR features above the level of detectivity. Thus our observations place definitive constraints on the hypothesis of Brecher *et al.*:

(1) The ring, if it exists in the equatorial plane between 2 and $5.5 R_{\odot}$, has an IR brightness lower than 10^{-7} W cm^{-2} sr^{-1} μm^{-1} at 2.2 μm . The excess emission observed at $4 R_{\odot}$ by Peterson and MacQueen cannot be ascribed to a 'stable' ring structure, because if the dispersion time for such a ring were of the order of 10^{13} yr we should have seen it in the present experiment.

(2) The present upper limit would also imply an upper limit to the total number of 10 km objects of $\sim 10^8$, based on the visual magnitude $m_v = +8$ to $+10$ for the individual objects as hypothesized by Brecher *et al.*

(3) If the ring resides at $R \geq 5.5 R_{\odot}$, the total mass in the ring is $\leq 3 \times 10^{25}$ g which in turn will limit the total number of 10 km objects (made of graphite with a density of 2 g cm^{-3}) to $\sim 3 \times 10^6$. If the ring consisted of less refractory materials and were located at distances greater than $5.5 R_{\odot}$ from the Sun, our experiment would not have detected it. If smaller particles with size <10 km, that are spiralling into the Sun, contribute significantly to the total IR emission of the solar corona at distances less than $5.5 R_{\odot}$, it is possible that such radiation may mask the excess emission arising from a ring of larger particles with a sharp outer boundary within $5.5 R_{\odot}$. But then the observation of an excess emission at times, makes the problem more enigmatic and interesting for future measurements.

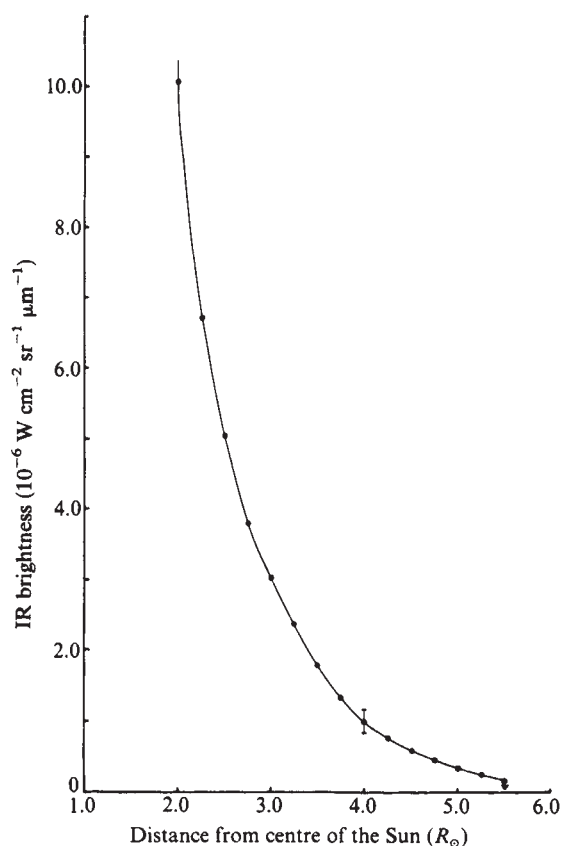


Fig. 1 The IR brightness of the solar corona in the equatorial plane from 2 to $5.5 R_{\odot}$.

If a ring structure exists around the Sun between 2 and 5.5 $R_{\odot}$ in the equatorial plane, this experiment places an upper limit of $10^{-7} \text{ W cm}^{-2} \text{ sr}^{-1} \mu\text{m}^{-1}$ for its emission at 2.2 μm . Further, observations seem to indicate a high degree of variability in the near IR emission from the solar corona.

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Temperature dependence for the power outputs of *n*-CdSe liquid junction cells

J. F. McCann, M. Skyllas Kazacos & D. Haneman

School of Physics, The University of New South Wales, PO Box 1, Kensington, New South Wales 2033, Australia

Thin film *n*-CdSe photoanodes have great potential as efficient and inexpensive electrodes in liquid junction photovoltaics^{1–3}. This stems from the stability of *n*-CdSe in the S/S^{2-} electrolyte, its direct band gap⁴ of 1.7 eV and the large barrier height, $\Phi_B(T)$, of ~ 1 eV at the *n*-CdSe- S/S^{2-} interface⁵. The recently reported 8% AM1 conversion efficiency for a cadmium chalcogenide thin film based cell⁶ suggests that these low cost devices may soon compete favourably with the conversion efficiencies of the more expensive single crystal solid state photovoltaics. In view of the increasing practical importance of this system, the study of any parameter which may affect its performance is relevant. We report here the dramatic effect of temperature on the power outputs of *n*-CdSe based cells.

Single crystals of *n*-CdSe (Cleveland Crystals Inc.) were fabricated into electrodes by forming an ohmic contact on the back side of each crystal with In:Hg amalgam and soldering onto a copper wire. The crystals were mounted in epoxy resin, polished and etched by the usual techniques¹ before use. Thin films of *n*-CdSe were prepared on $1 \times 3 \text{ cm}^2$ Ti strips by the electroreduction of Na_2SeSO_3 in the presence of a high concentration of Cd^{2+} (ref. 7). The films were annealed in a vacuum at

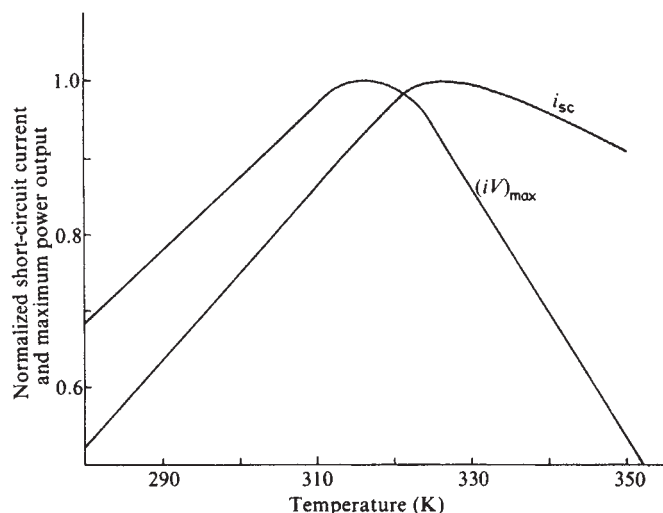


Fig. 2 The normalized short-circuit current and maximum power output of a thin film *n*-CdSe/S(1M)-Na₂S(1M)-NaOH(1M)-Se(0.7M)/CoS cell as a function of temperature.

400 °C for 1 h. Several CoS counter electrodes were prepared by the method described by Hodes *et al.*⁸. The electrolyte used in this study consisted of S(1M)-Na₂S(1M)-Se(0.07M)-NaOH(1M) and was prepared with AR grade reagents and distilled water. During all experiments the electrolyte was continuously purged with N₂ and stirred. Cell current-voltage (*i*-*V*) curves were obtained by varying a load resistor between the *n*-CdSe and CoS electrodes. A 300-W General Electric ELH lamp was used to illuminate the semiconductor electrodes by a Schott KG-2 filter (intensity $\sim 70 \text{ mW cm}^{-2}$).

The resistance of a cell with the electrodes separated by ~ 1 cm was measured by passing a sinusoidal voltage (Heathkit ET-3100 sine wave generator) of the desired frequency across two resistors (R_1 and R_2) which were in series with each other and with the cell. A PAR lock-in amplifier (model 124A) was used to measure the in phase signal, Z_R , differentially across R_2 (typically 1 Ω) and directly across the cell (resistance R_{cell}) which was kept at open circuit. The amplitude of the sine wave across the cell was kept < 10 mV by adjusting the value of R_1 . The value of R_{cell} was determined by comparing the voltage reading across R_2 with the voltage reading across the cell.

Series of *i*-*V* curves were determined for several *n*-CdSe cells over a range of temperatures. These were obtained by first cooling down the electrolyte in the single compartment cell, and gradually heating it on a heating mantle over a period of ~ 2 h. *i*-*V* curves were obtained at various temperature intervals and the electrodes were illuminated only while the curves were being recorded. To verify that the effects observed were due to temperature and not time, the solution was then allowed to cool down and *i*-*V* curves again recorded. The data obtained on cooling were equivalent to those obtained on the heating cycle. The open circuit voltages of both single crystal and thin film based cells dropped by ~ 2 mV per degree C as the temperature was increased, which is comparable with the decrease in V_{oc} typically observed in solid state photovoltaics for comparable illumination intensities. In the latter systems, this trend can be predicted from the relation for the open circuit voltage, $V_{\text{oc}}(T)$ as a function of temperature⁵.

$$V_{\text{oc}}(T) = \frac{nkT}{q} \ln \left[\frac{i_{\text{ph}}}{A^* T^2 \exp(-\Phi_B/nkT)} + 1 \right] \quad (1)$$

where q is the electronic charge, k is Boltzmann's constant, T is the absolute temperature, n is a factor which takes the non-ideal behaviour of the semiconductor into account, i_{ph} is the semiconductor photocurrent, and A^* is Richardson's constant for a given semiconductor. In the case of a photoelectrochemical cell, however, this relationship has to be modified to include the

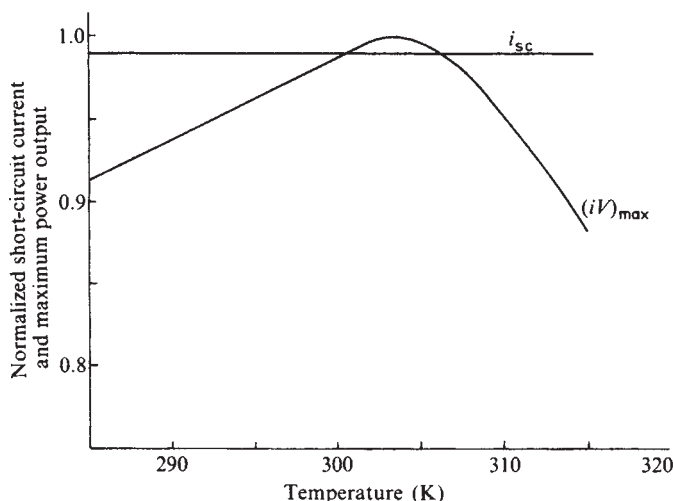


Fig. 1 The normalized short-circuit current, i_{sc} , and maximum power output, $(iV)_{\text{max}}$ of single crystal *n*-CdSe/S(1M)-Na₂S(1M)NaOH(1M)-Se(0.07M)/CoS as a function of temperature.

temperature dependences of the redox potential of the electrolyte, and the flat band potential of the semiconductor. These factors could result in a temperature dependence of the barrier height Φ_B . Variations in the semiconductor band gap and solution absorption with temperature could also have an effect on i_{ph} . The A^*T^2 term in equation (1) would also need to be modified to take the electron transfer probability through the Helmholtz layer into the electrolyte into consideration. In spite of these limitations of equation (1) when applied to a liquid junction photocell, the observed decrease in V_{oc} for n -CdSe in S/S^{2-} of 2 mV per degree C for increasing temperature is in agreement with the value predicted by this relation and could suggest that the modifications outlined above may not be very significant.

The typical effects of temperature on the short-circuit current and power outputs of both single crystal and thin film n -CdSe cells are shown in Figs 1 and 2 respectively. Although the short circuit current for the single crystal cell remains essentially constant over the temperature range, the power output of the cell exhibits a maximum at $\sim 30^\circ\text{C}$. This is due to a corresponding increase in the fill factor with temperature which counteracts the drop in V_{oc} and thus results in the maximum observed for the power output of the cell. The behaviour of the thin film cell shows a more dramatic temperature dependence. In contrast to the single crystal cells, the short-circuit current for this type of cell increases drastically up to about 50°C . Experiments on several thin film electrodes have indicated that the short circuit current tends to level off beyond 50°C rather than drop slightly as seen in Fig. 2. There is a corresponding improvement in the output power up to $\sim 40^\circ\text{C}$. However, due to the continuing decrease in V_{oc} and the levelling off of the short circuit current, the power output decreases rapidly at higher temperatures resulting in an optimum cell operating temperature of around 40°C . We suggest that the striking effect of temperature on the short circuit current for thin film cells can be explained in terms of the dissolution kinetics of sulphur formed at the photoanode. Because of the polycrystalline nature of these films the electrolyte penetrates the fine pores. As the oxidation of sulphide progresses, the sulphide ion concentration within the pores becomes depleted. Hence the dissolution of sulphur formed within the pores, which occurs according to the reaction:



is limited by the rate of diffusion of sulphide ions into the pores from the bulk of the solution and partial passivation of the electrode occurs. The rate of sulphur dissolution increases with temperature until it is no longer a limiting factor. This causes the corresponding increase in the short circuit current observed up to $\sim 50^\circ\text{C}$ (see Fig. 2). Films grown by another technique (D. Miller and D.H., unpublished results) also show the temperature behaviour described above.

As a further test of the above hypothesis, experiments were carried out over a range of light intensities on a CdSe film grown by the latter technique. The kinetics of sulphur dissolution would be expected to have a much less important effect at comparatively low light intensities. Measurements between 0 and 40°C showed that the rate of increase of the short circuit current with temperature dropped from 0.33 at a light intensity of $\sim 140 \text{ mW cm}^{-2}$ to 0.15 at 70 mW cm^{-2} to 0 at 0.5 mW cm^{-2} . This behaviour is therefore consistent with the sulphur dissolution hypothesis.

The effect of temperature on the electrolyte resistance was also examined as this may also influence the i - V characteristics of n -CdSe cells. Figure 3a shows the resistance of a n -CdSe/ S - S^{2-} -Se-OH $^-$ /CoS cell plotted as a function of frequency both in the dark and under illumination, and shows that the resistance levels off at frequencies $\geq 20 \text{ kHz}$. This graph also illustrates the decrease in the net resistance of the semiconductor on illumination which is apparently due to a reduction in the resistance of the space charge region and an increase in the photoconductivity of the semiconductor bulk.

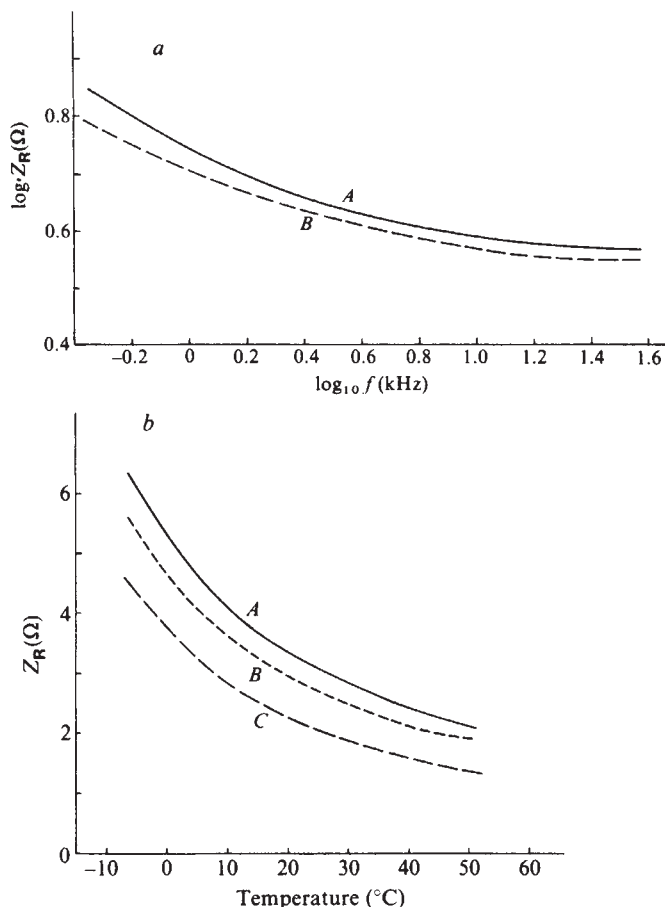


Fig. 3 a, The real component of the impedance, Z_R , of a thin film n -CdSe/ $S(1M)$ - $Na_2S(1M)$ - $NaOH(1M)$ - $Se(0.7M)$ /CoS cell plotted against frequency f (in kHz): A in the dark and B under illumination; $T = 22.5^\circ\text{C}$. b, Z_R at 28 kHz versus temperature for a thin film n -CdSe/ $S(1M)$ - $Na_2S(1M)$ - $NaOH(1M)$ - $Se(0.7M)$ /CoS cell: A in the dark and B under illumination. C, Pt-Pt cell in the same electrolyte.

Using an input frequency of 28 kHz, the above cell resistance was then measured as a function of temperature and compared with the results obtained using two platinum electrodes in the S/S^{2-} electrolyte. These results are presented in Fig. 3b. Again, the decrease in cell resistance on illumination of the CdSe can be observed in curves A and B. These curves also show the significant drop in cell resistance with increasing temperature. The parallel trend obtained with the two platinum electrodes (curve C) indicates that the temperature dependence is predominantly due to the electrolyte resistance rather than the semiconductor resistance varying as a function of temperature.

The above results therefore suggest that the changing resistance of the electrolyte as a function of temperature would be expected to influence the shapes of the cell i - V curves obtained for liquid junction solar cells. In fact, for the illuminated CdSe thin film solar cell used here, this resistance is seen to change from $\sim 4.6 \Omega$ at 0°C to $\sim 2 \Omega$ at 50°C . A significant increase in the fill factor of the cell i - V curves would be expected over this temperature range and was, in fact, observed in a previous study⁵. Another factor which may also influence the fill factor as a function of temperature is the change in the polarization of the CoS counter electrode with temperature. This latter effect was not examined in the present study but the Weizmann group have shown that polarization at a CoS counter electrode decreases with temperature¹⁰. This would also improve the fill factor of a cell with temperature.

The above results emphasize the importance of controlling the operating temperatures of n -CdSe liquid junction cells particularly those based on thin films to obtain the maximum possible photo-conversion.

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Zoned aluminium distribution in synthetic zeolite ZSM-5

R. von Ballmoos & W. M. Meier

Institut für Kristallographie und Petrographie, ETH Zürich, 8092 Zürich, Switzerland

ZSM-5, a prominent member of the new generation of high silica zeolites, is a potential catalyst. The activity of ZSM-5 type synthetic zeolites with respect to acid-catalysed reactions is a linear function of the Al content¹ which can assume extremely low values, but in catalytic applications is typically 1–3 Al per unit cell containing 96 Si + Al atoms. We now present substantial evidence that the Al distribution in ZSM-5 crystals is non-uniform. We first noted zoning of Al in ZSM-5 when examining scanning X-ray micrographs (Al K α) of 50 μ m crystals. The comparatively large crystals necessary for detailed measurements of Al concentration profiles using electron probe analysis suggest that Al is invariably concentrated in the rim portion of the crystals. No comparable zoning has been reported previously for zeolites.

ZSM-5 is synthesized in siliceous systems containing tetrapropylammonium (TPA) and sodium ions². The Si/Al ratio of ZSM-5 ranges from ~10 to >4,000 (ref. 1). Such figures refer to bulk composition of the crystals which typically do not exceed a few micrometres in size. The crystal structure of ZSM-5 (ref. 3) and the structural properties of its aluminosilicate framework have been described elsewhere^{4–6}. X-ray structure analysis could not be expected to provide information on the arrangement of the relatively few Al atoms and a uniform distribution has been assumed.

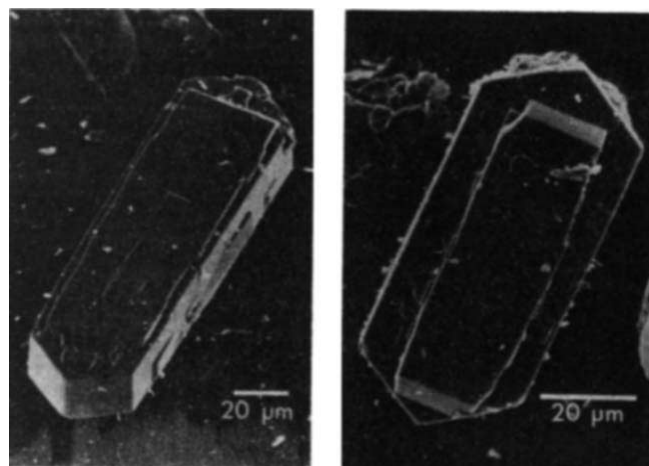


Fig. 1 Scanning electron micrographs of typical large ZSM-5 crystals. Gel particles are visible at the upper left.

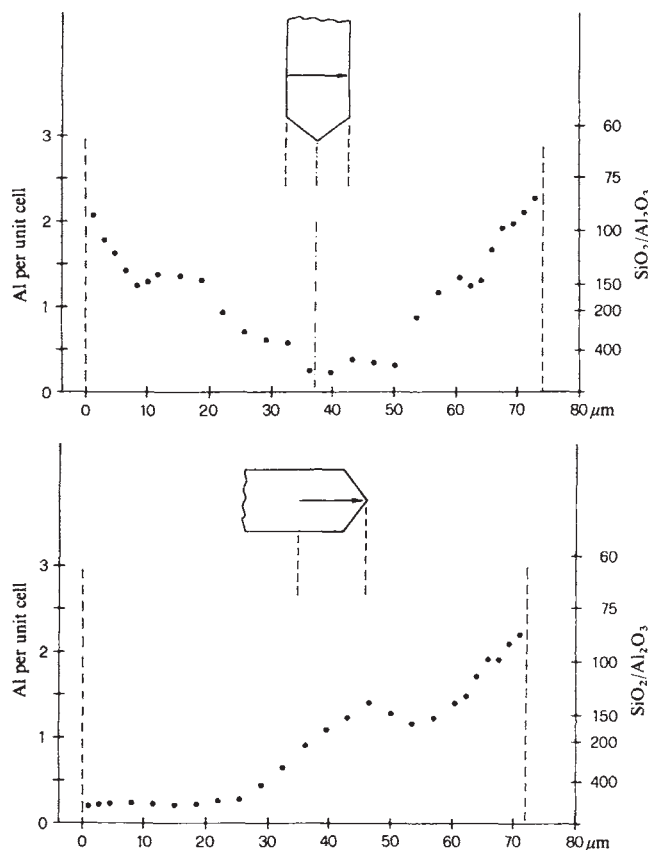


Fig. 2 Two perpendicular profiles of Al concentration across a section of a ZSM-5 crystal determined by microprobe step scans (shown in the inserted outline).

Appropriate conditions for growing large ZSM-5 crystals were determined, using dilute systems containing ~40–60 mg of solid per 10 cm³ of solution. A suitable molar composition is around 11SiO₂:Al(NO₃)₃:2NaOH:72(TPA)OH:3,300 H₂O. Crystallizations were carried out in small steel autoclaves (25 cm³) at 200 °C for 5 days⁷. The largest crystals obtained were 60–200 μ m. Figure 1 shows scanning electron micrographs of typical crystals which appeared invariably twinned. All solid products of the crystallizations contained a considerable amount of gel. The Si/Al ratio of ~3–4 for the gel contrasts strongly with that of the crystals with bulk Si/Al ratios of 50–100. X-ray powder diffraction patterns were used to identify the ZSM-5 crystals.

Five specimens were filled into holes of 4 mm diameter in an acrylate disk. The holes were then filled with an epoxy resin (Epotek 330) which was then cured overnight at 60 °C. Grinding was completed with 0–3 μ m diamond paste on a lapping wheel. The sample disks were cleaned with hexane and stored in water-free atmosphere. All samples were beryllium coated, and for some a very thin film of aluminium was first applied to assure better contact of the beryllium coating. Crystals of the silica end member of the ZSM-5 compositional series ('silicalite'⁸) were also prepared to monitor possible aluminium contamination. Electron microprobe scans used an ARL-EMX instrument at 15 kV, 0.08 μ A sample current, a beam diameter of 0.8 μ m producing an analysis volume of about 2 μ m³ and an average counting time of 8 s for each point, with crystal spectrometers (ADP and RAP). Orthoclase was used as a standard and the computer programs EMX and EMMA were used to process the data. Step advancement was mechanical at intervals of 1.8 or 3.5 μ m along a straight path. Only true cross-sections through the core of the crystal and oriented approximately parallel or perpendicular to *c* were analysed. Scans were taken crosswise for better centering.

A total of 60 scans were made of 40 large ZSM-5 crystals with representative Si/Al bulk ratios of 50–100. All Al concentration profiles showed pronounced zoning as in Fig. 2. The Al concentration in the core was invariably below that in the rim. Minimum and maximum concentrations of Al within a ZSM-5 crystal frequently differ by a factor of 5 or more. The most pronounced zoning, with Al concentration in the rim exceeding that in the core by $\gg 10$, was observed in Fig. 3. Core concentrations were often found near 0.2 Al per unit cell. In some crystals the Al concentration in the core was not above a detection level of 0.05 Al atoms per unit cell. Usually zoning is monotonic from a siliceous core to an Al-rich rim but some waviness (Figs 2, 3) could frequently be observed. (More details are presented elsewhere⁷.) All zoning was essentially symmetric.

Strong zoning of this kind has not so far been found in zeolites. There is a relative increase in the Si/Al ratios from core to rim of large NaX crystals of a few per cent⁹. This zoning in NaX is reversed and much less striking than that in ZSM-5. Crystals of natural faujasite, phillipsite and offretite were found to be uniform¹⁰. On the other hand, chemical zoning is present in feldspars of the plagioclase series¹¹.

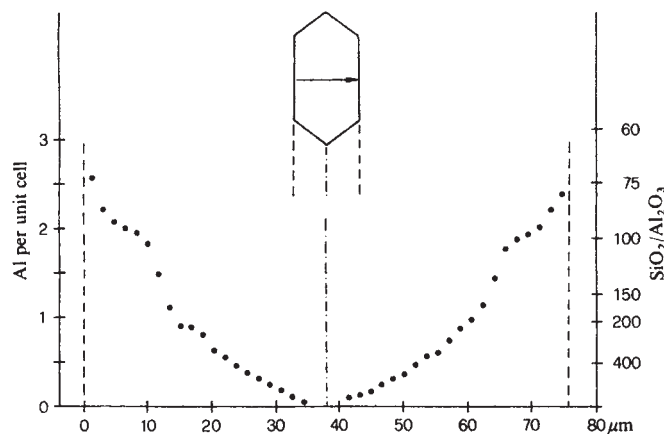


Fig. 3 Example of a scan which exhibits Al-zoning with extreme concentrations differing by a factor of more than 10. The innermost core concentration is around the detection limit of 0.05 Al per unit cell.

The presence of Al-rich solid gel in the products might suggest that the increased Al content in the rim is associated with dissolution of gel particles which enhances the Al content of the solution towards the end of crystallization. This would imply crystal growth from the liquid phase.

Although the zoning observed in large ZSM-5 crystals does not necessarily apply to much smaller crystals, their bulk compositions should be interpreted with caution.

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Volcanism and the dynamics of open magma chambers

Stephen Blake

Lunar and Planetary Unit, Department of Environmental Sciences, University of Lancaster, Lancaster LA1 4YQ, UK

The repeated eruption of many volcanoes is now accounted for by events taking place in a subterranean magma chamber, which is 'open' in the sense that the inflow of material to the chamber from below leads to a magma pressure P which exceeds the lithostatic pressure P_L by an amount greater than the tensile strength of the chamber walls (σ) before the contents of the magma chamber have reached thermodynamic and geochemical equilibrium. This is the condition which must be satisfied when an eruption occurs. We have now derived a relationship giving the critical volume of added material (ΔV) required to cause an eruption as a function of magma compressibility and the volume by which the chamber expands.

Geochemical, petrological and stratigraphic studies of lava piles at individual volcanoes have demonstrated that many sub-volcanic magma chambers are open systems where an evolutionary trend controlled by crystal fractionation is modified by the episodic influx of less evolved magma^{1–4}. Disequilibrium phenocrysts and glasses in some lavas and ejecta also show that magma mixing has preceded eruption^{5–10}. Such an open system of replenishment and eruption extends the lifetime of a chamber by buffering heat loss and is capable of chemically¹¹ and thermally¹² determining the evolution of the magma, depending on the fluid properties and dynamics of the magmas involved^{13,14}. In addition to their petrological significance, open magma chambers are important volcanologically. The inflation of volcanoes, often accompanied by seismic activity, is attributable to the influx of magma into a sub-volcanic storage system. Eruption begins when some critical amount of magma has accumulated causing the reservoir to burst^{15–19} and deflation typically accompanies withdrawal or eruption of magma²⁰.

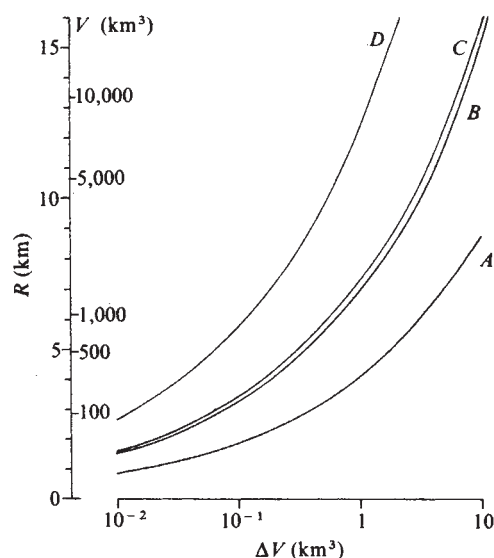


Fig. 1 The volume of magma, ΔV , which must be introduced into a chamber of constant volume, V_c , to trigger eruption according to $\Delta V/V_c = \sigma/b$. Also plotted is the radius, R , of a spherical chamber having volume V_c , but other geometries may be considered. Curves A and B, olivine basalt $b = 14$ GPa; C and D, andesite $b = 80$ GPa; A and C, strong volcano $\sigma = 50$ MPa; B and D, weak volcano $\sigma = 10$ MPa. For each case the critical values of σ/b are: A, 3.6×10^{-3} ; B, 6.25×10^{-4} ; C, 7×10^{-4} ; D, 1.25×10^{-4} .

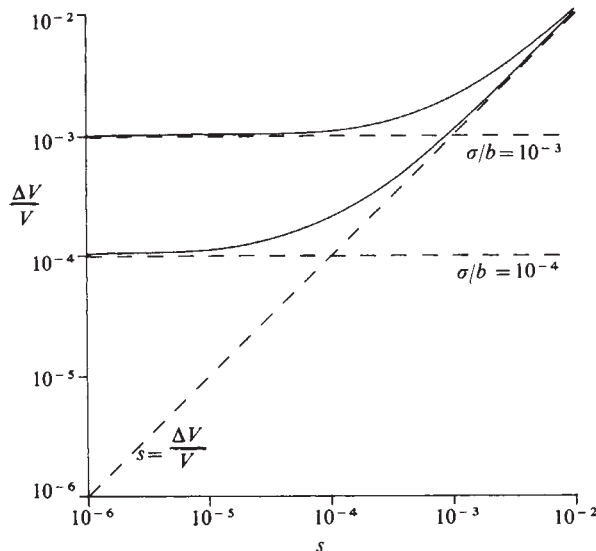


Fig. 2 Allowing a chamber to expand to a size $V_c(1+s)$ before eruption causes the critical ratio $\Delta V/V_c$ to increase according to $\Delta V/V_c = \sigma/b(1+s) + s$, which is shown by the heavy lines for the cases σ/b equal to 10^{-3} and 10^{-4} . When $s \ll \frac{\sigma}{b}$, $\frac{\Delta V}{V_c} \rightarrow \frac{\sigma}{b}$, approximately the case of a constant volume chamber; when $s \gg \frac{\sigma}{b}$, $\frac{\Delta V}{V_c} \rightarrow s$ and is independent of σ and b .

First, consider a constant-volume magma chamber which in equilibrium conditions (lithostatic pressure) contains a volume of magma V_c . When additional magma of volume ΔV is introduced, the contents of the chamber must become compressed to accommodate the new magma (an assumption which will be justified later). The compressed magma will occupy the volume V_c at a pressure P ($> P_L$), placing the chamber walls under tension. P and ΔV are related by the constitutive equation of the magma

$$V_c = (V_c + \Delta V) \exp [-(P - P_L)/b]$$

where b is the bulk modulus of the magma and ΔV is measured at P_L . The critical condition is arrived at when $P - P_L = \sigma$ which, for most rocks, lies in the range 10–100 MPa (ref. 21). The strength of volcanoes is, however, reduced by structural defects resulting from fragmental layers, vesicular and jointed lava flows, dyke and sill complexes and a generally stratified structure. Previous workers^{19,22,23} have used values for the tensile strength of volcanoes in the range 10–50 MPa. Values of b have been calculated from the seismic wave velocities and densities of rock melts measured by Murase and McBirney²⁴ and are found to vary from 14 GPa (Galápagos Islands olivine basalt, 1,200 °C) to 80 GPa (Mount Hood andesite, 1,000 °C). The relative magnitudes of σ and b (that is, $\sigma/b \sim 10^{-3}$) simplify the critical condition required for failure of the chamber walls to

$$\frac{\Delta V}{V_c} = \frac{\sigma}{b}$$

When the walls have failed, escape of magma is driven by decompression until magmatic pressure within the chamber has returned to lithostatic and only the stable volume of magma V_c is present. The expelled volume, V_e , is therefore equal to ΔV , as decompressional expansion on eruption is negligible.

Given the bulk modulus of a magma (which varies with composition and temperature) and the tensile strength of the volcano of interest, the erupted volume, being approximately equal to the volume required to trigger an eruption, is proportional to the size of the associated magma chamber.

Figure 1 depicts the relationship between ΔV required to trigger eruption from a spherical chamber of radius R and volume V_c . For basaltic volcanoes (Fig. 1, curves A, B) with

$0.01 \leq V_e < 1 \text{ km}^3$, chamber radii of 1–7 km are predicted, in good agreement with the sizes of exhumed gabbroic plutons²⁵, the seismically determined sizes of basic magma bodies in the upper crust²⁶ and the areal dimensions of calderas at strato-volcanoes. For a given chamber size and strength, the larger bulk modulus of cooler andesitic magma causes ΔV to be less than that required by a basaltic magma (Fig. 1, curves C, D). The range of V_e for both magma types is essentially the same, however, which therefore implies larger chambers at andesitic volcanoes ($R < 12.5 \text{ km}$) than at basaltic ones, a conclusion which seems to be substantiated, as dioritic and tonalitic plutons are often larger ($R < 15 \text{ km}$) than gabbroic plutons ($R < 5 \text{ km}$).

The assumption that V_c remains constant may not always be appropriate. If s is defined as the fractional volumetric expansion of the chamber, the critical condition for eruption becomes

$$\frac{\Delta V}{V_c} = \frac{\sigma}{b}(1+s) + s$$

Consequently, the critical ratio $\Delta V/V_c$ is dependent on the relative magnitudes of s and σ/b (Fig. 2). The amount of expansion which a given chamber experiences will depend on its host environment. In unfractured competent country rock, dilation of the chamber results from elastic deformation of the wall rocks. As the compressibility of solid rock is up to an order of magnitude less than that of silicate melts²⁴ the chamber volume will not be significantly increased and $s < \sigma/b$. Accordingly, newly introduced magma is most easily accommodated not by an increase in chamber volume, but by compression of the magma itself. In such cases the constant volume model as discussed above is a valid simplification and $\Delta V/V_c \approx \sigma/b = 10^{-3}$ adequately describes the critical condition for these eruptions (Fig. 1).

High degrees of chamber enlargement ($s > \sigma/b$) are thought to be due to the creation of new reservoir space by crack opening, filling and propagation such as in the rift zones of the Kilauea volcano^{27,28}. Conditions at Kilauea and many other mature volcanoes are favourable for this type of behaviour because the magma chamber resides in a readily fissured lava pile. During these intrusive episodes, surface deformation is manifest (refs 15, 29–31).

The effect on the critical condition of allowing chamber expansion (creation of additional storage space) is shown in Fig. 3 for the case $\sigma/b = 10^{-3}$ and $s = 0, 10^{-4}, 10^{-3}$ and 10^{-2} . When s exceeds σ/b , the factor controlling the onset of eruption (the value of ΔV) becomes the new size of the reservoir and is largely independent of σ/b . In these cases, the use of V_c and the

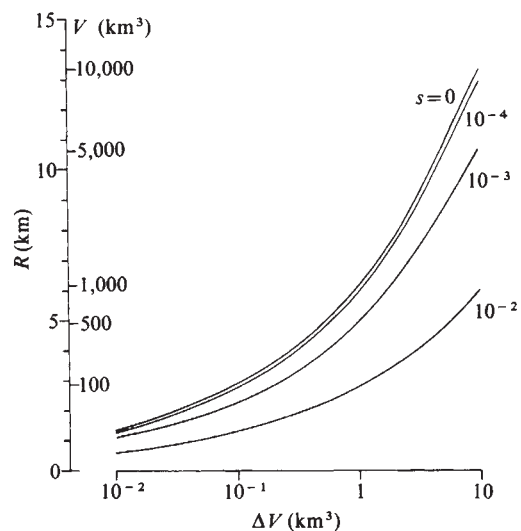


Fig. 3 The effect on ΔV of permitting chamber expansion $s = 0, 10^{-4}, 10^{-3}$ and 10^{-2} for chamber radius R , volume V_c and $\sigma/b = 10^{-3}$. When $s \geq \sigma/b$, chamber expansion causes a significant increase in ΔV for a given chamber size (see Fig. 2).

assumption of a constant volume condition ($s \sim 0$) would provide a large (up to an order of magnitude) overestimation of the stable chamber volume. For example, 10 km^3 of basaltic magma must be supplied to (and will later be erupted from) a 6.2-km radius chamber with volume $1,000 \text{ km}^3$ which has the ability temporarily to increase its storage capacity by as little as 1% (Fig. 3, $s = 10^{-2}$) whereas only 1 km^3 is required to trigger eruption from the same chamber if this additional space cannot be made available (Fig. 3, $s = 0$). Furthermore, caldera collapse or other tectonic effects will, by reducing the chamber volume to less than V_c , cause V_e to exceed ΔV . These effects have influenced activity in the Icelandic rift zones³². As V_c and particularly s , may vary over even a short span of a volcano's history, such tectonic influences will account for some of the variability between the amounts of magma erupted from the same volcano in successive eruptions.

Although basaltic magmas in plutonic conditions are generally believed to be undersaturated in volatile components, some eruptions may be related to chambers in which a free volatile phase has evolved. Because a gas is much more compressible than liquid magma, a given increase in P can be obtained by introducing a relatively small volume of magma or a relatively large volume of vapour. The critical value of ΔV is therefore virtually unaffected by the presence of a gas phase. Eruptions which are triggered by magma mixing¹⁰ are therefore deduced to be triggered solely by the increased fluid pressure resulting from injection of (ΔV) new magma and not by any vesiculation process. Basalt–rhyolite mixed magmas have been erupted irrespective of the water content of the rhyolitic magma. Although the rhyolitic liquids involved in the Askja 1875 and Novarupta 1912 mixed pumice eruptions are believed to have been at or near saturation with H_2O contents of 3 and 5–6 wt%, respectively^{33,34}, much drier mixed lava flows are not uncommon⁶.

The mechanisms discussed above serve as a general model on which to base concepts of stratovolcano development and seafloor spreading rates. Active periods, separated by repose periods of less than ten to hundreds of years and characteristic of a given volcano³⁵ are regarded as being associated with the uprise of magma at rates of several cubic metres per second over periods lasting up to several years, during which the critical amount of magma may be accumulated and eruption triggered more than once. As $\Delta V \approx V_c$, volcano growth rates are equivalent to the upward magma flux rate beneath the volcano which is in turn governed by magma generation, accumulation and uprise dynamics. When related to an absolute time scale, the evolution and growth of stratovolcanoes and mid-ocean ridges are a potential tool with which to examine these magmatic processes^{1–4,36–39}.

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Seismic quiescence in the Western Hellenic Arc may foreshadow large earthquakes

M. Wyss

CIRES, University of Colorado, Boulder, Colorado 80309, USA

M. Baer

Institute of Geophysics, ETH-Hoenggerberg, CH-8093 Zürich, Switzerland

South of Crete, the seismicity rate was constant in space and time between 1950 and 1978, but in the Western Hellenic Trench the rate was the same as that south of Crete until 1962, whereupon it decreased by 80%. We now suggest that the anomaly constitutes a warning of large earthquakes within the coming decade.

Studies^{1,2} of the events preceding 20 earthquakes of magnitude greater than 6.0 have shown decreases of about 50% in the frequency of small earthquakes for several years before the main shocks. This was for example the case in the years preceding a Hawaiian earthquake of magnitude 7.2, where the decrease in seismicity was accompanied by two other precursor phenomena—relaxation of crustal strain and a change of seismic velocity³. Our hypothesis is therefore that a significant decrease of seismicity rate within a given volume is a sign that preparations have begun for one or more major earthquakes within that volume.

The region surrounding the Aegean Sea has the highest seismicity rate in western Eurasia⁴, and its tectonic complexity has been widely discussed^{5–11}. There is general agreement that the Hellenic trench–island arc system is a clear tectonic unit, that subduction occurs along it and that it can be regarded as a plate boundary. The direction of convergence is roughly perpendicular to the Western Hellenic Arc and approximately parallel to its eastern segment⁶. The rate of convergence may increase from west to east and is estimated to be between 2.5 and 7 cm yr⁻¹ (ref. 6). Assuming that no substantial aseismic creep occurs, these consumption rates imply that enough strain energy has been stored here during loading cycles of the order of 100–200 yr to produce large earthquakes.

The time of the last very large ruptures along the Hellenic plate boundary is a matter of debate. Eight earthquakes of magnitude ≥ 7.75 occurred in this area between 1805 and 1926, but most authors consider that these events occurred at depths of 100 km and locations about 100 km north of the plate boundary¹². By this interpretation the plate boundary has not been ruptured in great events for about 200 yr, which would imply that 5–15 m of potential displacement have been stored. However, we propose the data on the destruction¹⁸ caused by these large earthquakes to mean that these events were shallow ruptures of the plate boundary²⁰. According to our interpretation, all the Hellenic plate boundary, except a 150-km segment west of Crete, was ruptured between 1805 and 1926.

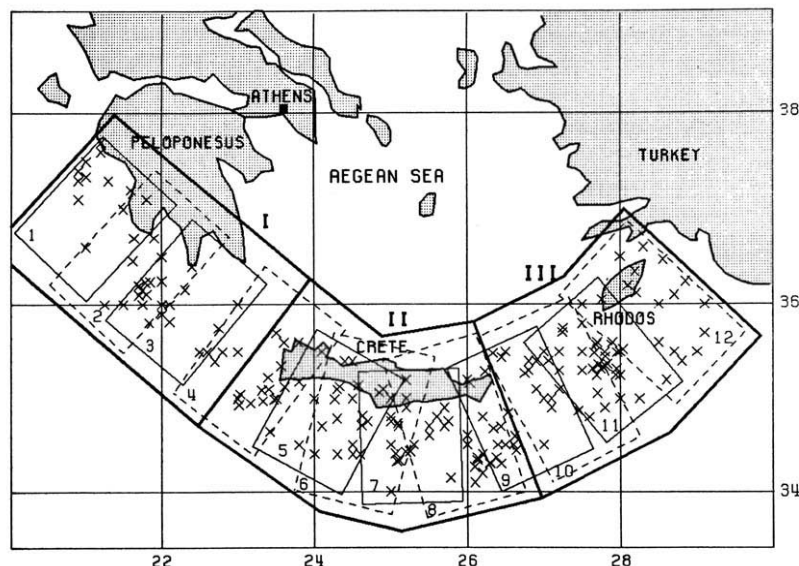


Fig. 1 Epicentre map of all earthquakes used (1950-78, 5.0 mag, $0 \leq \text{depth} \leq 100$ km) in the seismicity study of the Hellenic arc. Subvolumes for which seismicity rates as a function of time were determined separately are labelled 1-12. Large segments of the arc for which cumulative seismicity is shown as a function of time in Fig. 2 are labelled I, II and III.

We therefore estimate the potential earthquake slip stored at present to be ~ 2.5 -6 m (depending on the location along the arc). We conclude that, according to either interpretation, the Hellenic arc could reasonably be expected to be capable of very large earthquakes at the present time.

To determine whether any part of the arc is in a preparatory stage to rupture we studied the rate of occurrence of shallow earthquakes (0-100 km depth) as a function of space and time. A complete, pooled¹³⁻¹⁵ data set for earthquakes of >4.9 mag was obtained for the period 1950-78. Analysis of cumulative numbers of earthquakes as a function of time showed that their overall rate of incidence in the Hellenic Arc decreased slightly after 1962. To determine the portion of the plate boundary responsible for this change we examined seismicity rates within 12 random overlapping volumes, defined in Fig. 1. Three facts became evident: (1) The seismicity rates in the neighbouring volumes 5 6 7 and 8 were the same to within 10%; they were approximately constant over the 28-yr period; and they were relatively high ($1.5 \text{ events yr}^{-1}$) for this island arc. (2) Volumes 1-3 showed a common pattern of high rates ($\sim 1.5 \text{ events yr}^{-1}$) for the first 12 yr followed by a much lower rate ($0.5 \text{ events yr}^{-1}$) during the remainder of the period. (3) A mixture of rates and patterns existed in volumes 9-12. Based on these observations, subvolumes of the arc with similar patterns were merged for further study of the seismicity rates.

Figure 2 shows the cumulative numbers of earthquakes as a function of time for the super-volumes designated I, II and III in Fig. 1 which are of similar dimensions (Fig. 2). The seismicity rate in these three volumes was the same up to the early 1960s, after which time volume II continued to produce earthquakes at the same rate as before, while volume I showed a highly significant decrease of seismicity to about 20% of its previous rate. We interpret this decrease of seismicity to be an anomaly precursory to one or several large earthquakes in volume I. The data of volume III are less clear, but an anomaly may have existed there since about 1970. We conclude that the greatest likelihood of very large earthquakes exists in the western part of the Hellenic Trench.

The largest length of the arc segment which may be ready for rupturing is approximately 400 km (volume I and immediately adjoining volumes containing high seismicity). Based on the destruction caused by the great earthquakes which occurred in the Western Hellenic Trench during the nineteenth and early twentieth centuries¹⁸, we estimate the most likely length of future ruptures to be about 100 km. Using the hypothesis that the seismicity anomaly in the Western Hellenic Trench represents a precursor, and assuming that the precursor time is correlated with anomaly dimension, we expect that the sequence of large earthquakes will start between 1980 and 1991. Using seismicity data alone, we cannot estimate the time of occurrence

more accurately than this because of the scatter in the data defining the anomaly dimension versus precursor time relation¹.

We expect an earthquake with the following source parameters to rupture the Hellenic arc plate boundary: location, near 23.5°E ; size, lengths = 100 ± 50 km, magnitude 7.75 ± 0.5 ; occurrence, $1980 \leq T_0 \leq 1991$.

Although precursor seismicity patterns cannot be identified with certainty along the other segments of the Hellenic Trench, it seems reasonable to expect large earthquakes there also. However, this prediction should not be made without emphasizing the rudimentary nature of our understanding of the processes which lead to earthquakes. Even though the number

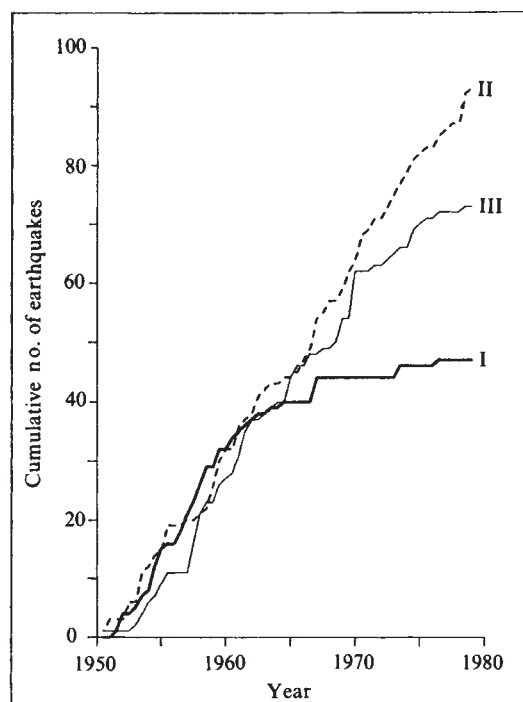


Fig. 2 Cumulative number of earthquakes (≥ 5.0 mag) as a function of time for the three large segments I, II and III of the Hellenic arc (defined in Fig. 1). As the seismicity in volume III is dominated by swarms (steps in the curve), we can reach no conclusion for this region. Note that the seismicity rates in I and II are closely similar up to 1962, after which time the rate in volume I is reduced to 20% of this rate while the rate in II continues at the pre-1962 level. The establishment of the World Wide Standard Seismograph network did not affect seismicity resolution in the area because the location capability is mostly based on European stations.

of well documented seismicity quiescence precursors is increasing, we do not know whether it is possible for seismicity quiescence anomalies to be terminated without a main shock.

Nevertheless, we believe that precautionary measures are warranted, although we are aware of the possible negative effects of broadcasting an earthquake prediction¹⁹. Ohtake *et al.*¹⁶, about 2 yr before the 7.8 mag Oaxaca earthquake in 1978, showed that quiescence had begun in the Oaxaca gap in mid-1973, but their report produced no constructive action and was, in fact, ultimately detrimental to the area concerned. We hope that previous mistakes will not be repeated in the case of the Hellenic Trench and that scientific surveillance of the area will be started.

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Miocene spreading centre south of Isla Guadalupe

Rodey Batiza

Department of Earth and Planetary Sciences and
McDonnell Center for Space Sciences, Washington University,
St Louis, Missouri 63130, USA

Clement G. Chase

Department of Geology and Geophysics, University of Minnesota,
Minneapolis, Minnesota 55455, USA

The Juan de Fuca, Rivera, Cocos and Nazca plates in the eastern Pacific are small fragments remaining from the fragmentation and partial subduction of the long, narrow Farallon Plate which began as early as 55 Myr ago¹. This fragmentation apparently resulted from interaction of the very irregular Pacific–Farallon ridge with the uneven coastlines of the American Plate^{1–4} and involved the formation of ridge–trench transforms⁵, creation of new spreading centres⁶, ridge jumps^{1,7,8}, deactivation of subduction zones² and other phenomena¹. We present here magnetic evidence that a fifth small fragment of the Farallon plate is preserved in the eastern Pacific off the coast of Baja, California. Its preservation is the result of an eastward jump of the Pacific–Farallon ridge ~12.5 Myr ago which was preceded by asymmetric spreading and perhaps pivotal subduction¹. Menard¹ first suggested this idea and showed that anomaly 5B is symmetric and parallel to a linear trough trending southwards from Guadalupe Island. He named the small fragment of the Farallon plate preserved between this trough and the margin of Baja California to the east, the Guadalupe plate¹. We show that both anomalies 5B and 5C symmetrically flank the Guadalupe trough. Our results and conclusions are not based on new magnetic data, but rather on reinterpretation of aeromagnetic profiles published by Taylor *et al.*⁹.

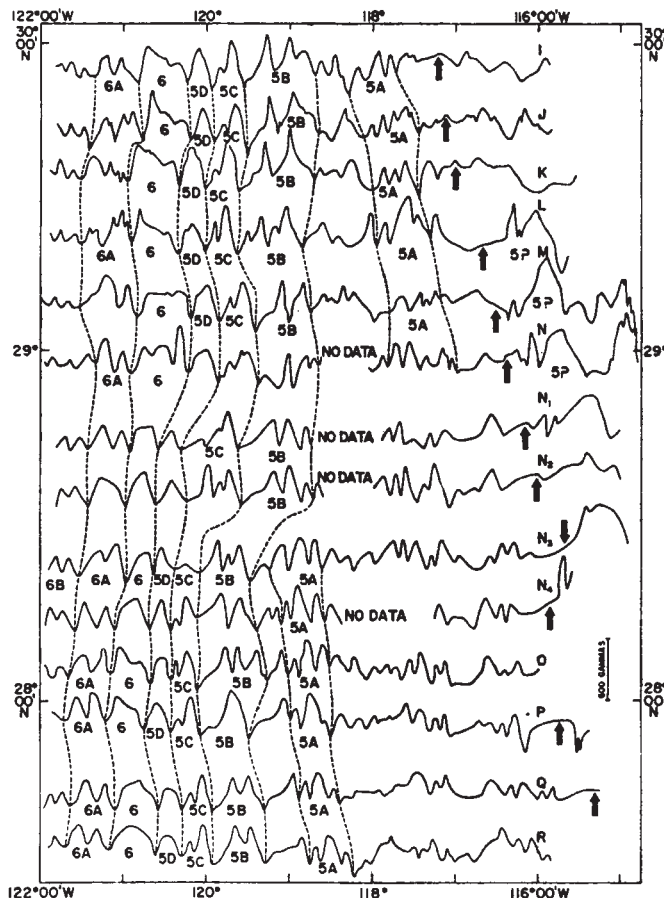


Fig. 1 Original data and interpretation of Taylor *et al.*⁹ reproduced exactly. Arrows indicate the location of the 1,000-fathom bathymetric contour.

Figure 1 shows the data of Taylor *et al.*⁹. This area of the eastern Pacific was first studied by Krause¹⁰ who recognized lineated magnetic anomalies east of Isla Guadalupe. Later studies^{2,11} confirmed their presence, but as Atwater² states "near Guadalupe Island, (the magnetics) lack distinctive character and thus remain unidentified except that they probably belong to the anomaly 5B–5A sequence." Menard¹ was apparently confident that anomaly 5B could be identified there but his interpretation has remained controversial. In our interpretation of the magnetic anomalies of the area we rely on closely spaced east–west aero magnetic anomaly profiles⁹ which are shown together with their original interpretation in Fig. 1. These data allow the anomalies in the unevenly preserved anomaly 5 series to be identified more confidently than before.

Taylor *et al.*⁹ emphasized the variable seafloor spreading rates in the vicinity of Isla Guadalupe. In their interpretation, spreading rates decrease monotonically from the latitude of Isla Guadalupe northwards and increase southwards although remaining at uniformly lower rates than north of the island. Their suggestion that a fracture zone is present at about the latitude of Isla Guadalupe has been confirmed by seismic refraction work¹². However, Taylor *et al.*⁹ did not attempt a detailed reconstruction of the tectonic history of the area.

We have renumbered the magnetic anomalies of Taylor *et al.*⁹ in accordance with a more recent magnetic time scale and anomaly nomenclature¹³. Figure 2 shows our new anomaly identifications which differ from those of Taylor *et al.*⁹ (Fig. 1) by the addition of anomaly 5E and the shift of the rest of the anomaly 5 series. We have also reinterpreted some of the profiles south of Isla Guadalupe and have identified reversed anomalies 5B and 5C to the east of Guadalupe (Fig. 3). Finally, we have plotted the magnetic anomalies of Fig. 1 onto Taylor *et al.*⁹ map of the area to show the geographical distribution of

Table 1 Average spreading rates

	6A-5E (West)		5D-5C (West)		5C-5B (West)		5B-12.56-5B (Fossil ridge)		5B-5C (East)	
	Profile	Rate (mm yr ⁻¹)	Profile	Rate (mm yr ⁻¹)	Profile	Rate (mm yr ⁻¹)	Profile	Rate (mm yr ⁻¹)	Profile	Rate (mm yr ⁻¹)
North segment	CC	30								
	FF	31	B	50						
	B	32								
	D	32	D	50	D	50?				
	G	33								
Middle segment	K	35	K	48?	J	46				
	M	34	L	46?	K	46				
			M	48?						
South segment	N ₂	33	N ₂	40	N ₃	43	N	34	N ₂	46
	O	30	N ₃	40	O	41	N ₂	34	O	44
	P	30	O	40	Q	39	N ₃	36	Q	45
	Q	30	R	41	R	41	N ₄	35?		
							Q	33		
							Q	32		

Profile letters correspond to those of ref. 9, some of which are shown in Fig. 1. North, middle and south segments are shown in Fig. 4. Spreading rates were estimated by comparing data profiles with synthetic profiles of known spreading rate, therefore estimates of precision of these values are subjective, and are no better than ± 1 mm yr⁻¹.

the magnetic anomalies (Fig. 4). Table 1 shows average spreading rates for various time intervals computed using Blakely's time scale for the Miocene¹³.

Figure 4 indicates important aspects of the tectonic evolution of the area. First, we have detected a major offset in the anomaly 5 series north of Guadalupe (Fig. 4) which corresponds to a

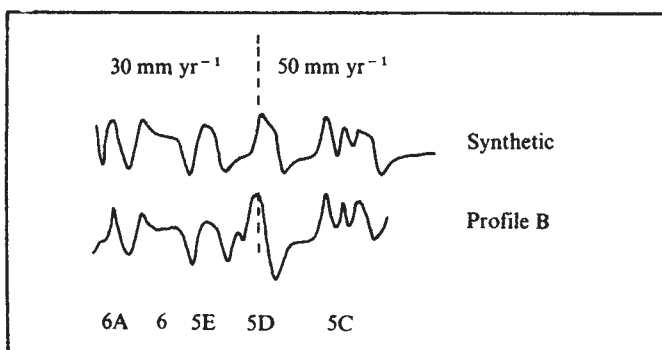


Fig. 2 Comparison of synthetic anomaly profile with data of profile B. Our new identifications are shown. Note variable spreading rate of synthetic profile.

series of east-west ridges. The magnitudes of the offsets of anomalies 5E, 5D, and 5C increases monotonically as they do for the fracture zone at the latitude of Isla Guadalupe. Several smaller anomaly offsets may also exist in the region north of the island, but more closely spaced magnetic data will be needed to confirm their presence. Figure 4 represents the simplest possible anomaly pattern which can be derived from the data.

The tectonic history of the region inferred from this pattern of anomalies is: (1) During anomaly 6A and 6 time (20–22.3 Myr BP) two segments of the Pacific–Farallon system offset by a transform fault at about the latitude of Isla Guadalupe were spreading at maximum rates of ~ 35 mm yr⁻¹ (Table 1). The rates for the northern segment increase southwards producing fanning anomalies and an arcuate fracture zone. For this segment, rates increase from ~ 30 to 33 mm yr⁻¹ in the south. Anomaly 6A shows little or no offset, but anomaly 6 is offset across the fracture zone; thus, either spreading was asymmetric or the northern and southern segments belonged to different plates. We prefer the first explanation.

(2) Between anomaly 5E and 5B (12.5–20 Myr BP) reorganization of spreading geometry resulted in formation of a third ridge segment north of Isla Guadalupe. This may have

been caused by interaction of this portion of the Farallon plate with the continental margin of Baja California. Anomaly offsets across this transform fault–fracture zone increased monotonically with time and spreading rates were variable (Table 1). Spreading rates south of Isla Guadalupe increased to about 40 mm yr⁻¹ as shown previously⁹, but then slowed again to about 35 mm yr⁻¹.

(3) Just after anomaly 5B time (12.5 Myr BP) the southernmost segment of the Pacific–Farallon system in this area jumped to the east. This is inferred from the presence of anomalies 5B and 5C east of the Guadalupe trough, which we suggest is an extinct spreading centre. This series of discontinuous linear troughs (Fig. 4) extends from the southern tip of Isla Guadalupe to the Shirley Trough at about 27° N (J. Mammerickx, personal communication). Table 1 shows that during anomaly 5C and 5B time, spreading at the Guadalupe Trough was asymmetric: about 41 mm yr⁻¹ on the west flank and 45 mm yr⁻¹ on the east. This is why we favour asymmetric spreading over plate

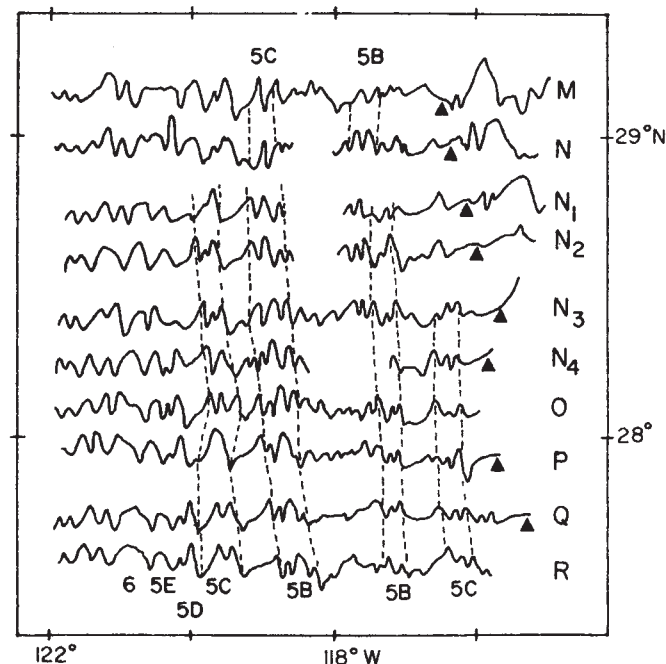


Fig. 3 Dashed lines show our new magnetic anomaly identifications and correlations for the southern part of the area which includes the Guadalupe Trough. ▲, Location of the 1,000-fathom bathymetric contour.

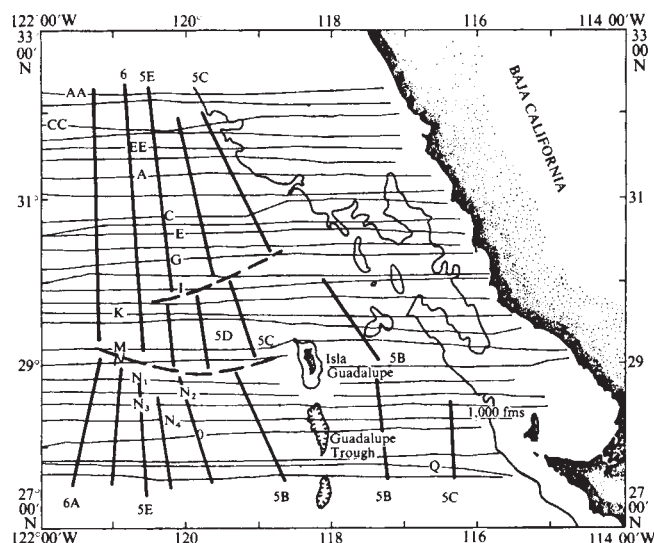


Fig. 4 Our new magnetic anomaly interpretations and the Guadalupe Trough¹⁴ added to one of Taylor *et al.*'s maps.

fragmentation as an explanation for the smoothly increasing magnetic anomaly offsets on both transform fracture zone systems in the area.

To test our new magnetic anomaly interpretation east of Isla Guadalupe as well as the hypothesis of interrupted spreading (ridge jump), synthetic magnetic profiles for continuous versus interrupted spreading are compared with data of Taylor *et al.*⁹ in Fig. 5. While neither synthetic is a perfect match to the data, the interrupted spreading model is certainly a better fit.

It is not known whether the ridge segments north of Guadalupe also jumped to the east at the time when the Guadalupe Trough became inactive because linear magnetic anomalies farther east have not been recognized. If Taylor *et al.*⁹ are correct in their identification of anomaly 5 in profiles L, M and N then these segments probably continued to spread continuously (at least until 9 Myr BP). Alternatively, Taylor *et al.*'s⁹ anomaly 5 could be 5A, or a buried basement ridge, or perhaps even another fossil ridge crest. In the former case, Isla Guadalupe occupies the intersection of a fracture zone and a fossil ridge, and is not simply built along a fossil ridge as previously proposed¹⁴. More detailed magnetic data will be required to determine the precise location of this fracture zone and thus the actual tectonic environment in which the Guadalupe volcanoes originated and evolved.

The spreading rate data of Table 1 give an insight into the possible relationships between the observed systematic changes of spreading rate before the ridge jump and the underlying

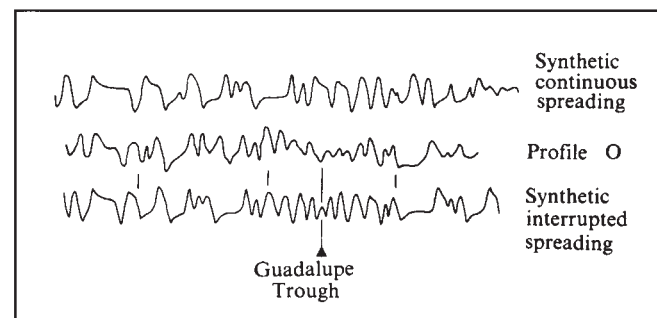


Fig. 5 Comparison of profile O data to both continuous and interrupted spreading synthetic anomaly profiles. The interrupted spreading synthetic is a composite of four profiles generated at various spreading rates (segments separated by short vertical lines above the profile). The spreading rates for these segments are: 30 mm yr⁻¹ (west), 40 mm yr⁻¹, 33 mm yr⁻¹ and 44 mm yr⁻¹ (east). Although neither synthetic is a perfect match to the data, interrupted spreading is a much better fit.

causes of such jumps. As shown above, the ridge jump was probably preceded by at least 10 Myr of asymmetric spreading in the entire region. Spreading rates increased from 30–33 to 40–50 mm yr⁻¹ between anomaly 5E and 5D time (18–19 Myr) and remained high but variable until just before the ridge jump when spreading rates again slowed to ~34 mm yr⁻¹. While the underlying causes of such jumps are not yet well understood, clues to the mechanism for ridge jumps may be provided by detailed study of the spreading history of a ridge segment before episodic migration. In the case of the Guadalupe trough, the slower spreading, its asymmetry and the interaction between the farallon plate and the continental margin of Mexico may all have contributed to the ridge jump. Alternatively, the spreading history of the Guadalupe trough may reflect the action of different causes of ridge migration, for example, relative displacement between elements of mantle convection and the overlying lithosphere.

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Vertical distribution of mercury in sediments from the East Pacific Rise

Malcolm E. Cox & Gary M. McMurtry

Hawaii Institute of Geophysics, University of Hawaii, Honolulu, Hawaii 96822, USA

Concentrations of mercury are often high in waters and sediments associated with volcanic hydrothermal systems, in both marine and terrestrial environments. Whereas the concentration of mercury in open ocean waters is reported to be in the range 0.002–0.05 µg l⁻¹ (refs 1–4), significantly higher values of up to 1.4 µg l⁻¹ are reported in waters near, or having circulated through, areas of submarine hydrothermal activity^{2,5}. Mercury concentrations of up to 400 p.p.b. (parts per 10⁹) on a carbonate-free basis (CFB) have been measured in surface sediments on the East Pacific Rise crest, but concentrations decrease to <20 p.p.b. (CFB) on the peripheral seafloor⁶. Similar high concentrations from 160 to 890 p.p.b. (CFB) have been recorded in near-surface sediments from the median valley of the Mid-Atlantic Ridge⁷. We report here on the vertical variations of mercury in sediments from the East Pacific Rise, and suggest that increased concentrations are correlated with periods of increased submarine volcanic activity.

Detailed down-core mercury analyses were made at 10-cm intervals on three piston cores collected by the RV Kana Keoki in the vicinity of the East Pacific Rise. Two of the cores, KK71-PC85 and KK71-PC87, located within 40 km of each other, are from within 50 km of the palaeomagnetically defined spreading axis of the East Pacific Rise at 6°S latitude; the third, KK72-PC8, is from the adjacent Bauer Basin (Fig. 1). These analyses were conducted to observe what vertical variations may occur in mercury concentration in marine sediments, knowing

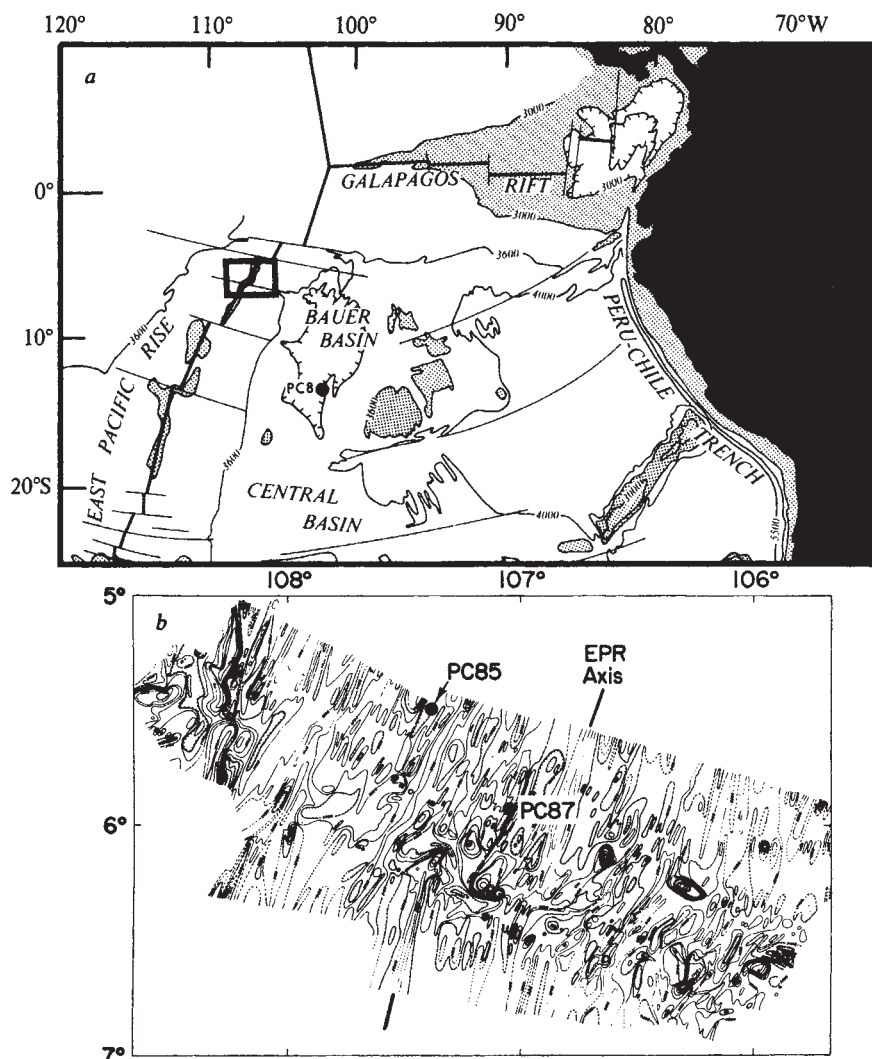


Fig. 1 *a*, General bathymetric map of the Nazca plate, south-east Pacific, showing major physiographic features and core locations. Box indicates detail map (*b*). Bathymetry after ref. 29. *b*, Detailed site survey area of the East Pacific Rise at 6° S latitude. Bathymetry after ref. 30.

that (1) both terrestrial and submarine volcanism are episodic in nature; (2) temporal variations in bottom water mercury anomalies over spreading centres have been observed⁸; and (3) most published sediment mercury analyses are from the surface or near-surface.

Samples were immediately taken from the split core sections and were individually sealed in plastic vials before analysis on a

Jerome Instrument Corporation model 301 gold film mercury detector. Weighed, air-dried samples were heated to >800 °C, and the mercury vapour released was transported by Hg-free carrier gas through various filters to remove water and acid vapours before adsorption onto the gold film. Changes in the resistance of the gold film due to mercury adsorption were monitored and calibrated by injections of standard amounts of

Table 1 Down-core chemical composition

Core no.	General lithological description	Sedimentation rate* (cm kyr ⁻¹)	CaCO ₃ † (wt %)	SiO ₂ (wt %)	Fe ₂ O ₃ (wt %)	MnO (wt %)	Al ₂ O ₃ (wt %)	Cu (p.p.m.)	Zn (p.p.m.)	Hg‡ (p.p.b.)
KK71-PC85	Radiolarian-rich, nannofossil foram ooze	2.4 ± 0.3	82.9 ± 3.3	12.7 ± 2.9	2.00 ± 0.62	0.17 ± 0.05	0.24 ± 0.13	268 ± 64	36 ± 16	58 ± 44
						<i>n</i> § = 12				<i>n</i> = 48
KK71-PC87	Clay, radiolarian and diatom bearing, foram-rich, nannofossil ooze. Minor ash bands throughout core	1.4 ± 0.1	81.3 ± 4.1	10.7 ± 1.7	2.85 ± 1.23	0.43 ± 0.35	0.47 ± 0.61	268 ± 42	34 ± 14	29 ± 29
						<i>n</i> = 18				<i>n</i> = 80
KK72-PC8	Nannofossil bearing, ash-rich clay	0.41 ± 0.06	1.39 ± 0.14	30.9 ± 4.1	27.7 ± 3.8	7.25 ± 1.24	8.28 ± 0.84	1061 ± 97	113 ± 24	0.7 ± 1.8
						<i>n</i> = 22				<i>n</i> = 42

* Determined by excess ²³⁰Th method using γ-ray spectrometry¹⁷.

† CaCO₃ determined by gas volume method; all others by X-ray fluorescence spectrometry. Precision for CaCO₃ ranges from 1 to 6%; all others within 6% except Cu (15%) and Zn (50%) (ref. 27).

‡ Hg analyses performed by gold-film mercury analyser⁹ which compares with analyses by atomic absorption²⁸. Precision within 5%. Accuracy within 10%.

§ No. of samples.

mercury saturated air⁹. The accuracy of the procedures was monitored with a soil standard (USGS geochemical exploration sample no. 5) of known mercury concentration. General lithological descriptions, sedimentation rates and mean down-core chemical compositions based on samples taken at 50-cm intervals are presented along with the mean down-core mercury results in Table 1. Figure 2 shows plots of mercury concentration versus depth for the East Pacific Rise sediment cores.

The extreme vertical variations in mercury concentration in the East Pacific Rise sediments are significant, and may demonstrate episodic submarine volcanic activity. In agreement with previous work⁷, the variations in mercury show little correlation to variations in other sediment components. By using an approximate sedimentation rate of 2 cm kyr^{-1} , and assuming that each major mercury peak represents an episode of significant submarine volcanic activity and that mercury concentrations are not appreciably affected by variable bottom-water movements, we can calculate mean recurrence intervals of the order of 25,000–35,000 yr for this region of the East Pacific Rise. These intervals agree within factors of two or three with periods separating major cycles of spreading activity ($\sim 10,000 \text{ yr}$) suggested from geological evidence for the FAMOUS area, the Galapagos Rift¹⁰ and the East Pacific Rise at 21° N latitude¹¹. However, this correlation further assumes that rise crest volcanic activity is confined to the spreading axis, and does not consider such activity or hydrothermal emission from nearby faults and seamounts (Fig. 1) which may complicate the observed vertical distribution of mercury. The lack of correspondence between major mercury peaks in the two cores and the generally higher concentration in core KK71-PC85, which is more distant from the spreading axis, suggest that the volcanic activity responsible for the mercury concentrations may be localized. More detailed sampling will be needed to establish the spatial and temporal relationship between volcanic activity and sediment mercury concentration. These measured variations in

mercury concentration are also important in illustrating that surface values alone are not an adequate representation of the mercury concentration at a given location.

The absence of mercury in the Bauer Basin core agrees with previous surface work in this region⁶. Of the 42 analyses over the topmost 3.5 m of core KK72-PC8, only seven were above the 1 p.p.b. detection limit (Table 1). The basin is characterized by sediments rich in metals^{12–14}, and anomalously high heat flow has been measured there¹⁵. Various origins for the metals in these sediments have been proposed, including transport of hydrothermally-derived material into the basin from the adjacent East Pacific Rise¹⁶, and hydrothermal activity within the Bauer Basin^{14,15}. If a hydrothermal origin is feasible, then the fixation process for mercury in these sediments must be different from that for East Pacific Rise sediments. We propose that this difference results from a higher oxidation potential caused by the relatively lower sedimentation rates in the basin (Table 1 and ref. 17). About 80% of the inorganic mercury in oxic seawater will exist as the highly soluble HgCl_2 form¹⁸, whereas aqueous mercury will exist as the less soluble Hg^0 or HgS_2^{2-} forms in less oxic waters^{19,20}. This oxidation-reduction behaviour may also apply to organically complexed forms of mercury. Approximately 30% of the total aqueous mercury in seawater has been shown to associate with organic matter²¹, and it is reasonable to assume that such associations will also occur in pelagic sediments. Preservation of organic matter in pelagic sediments is a function of the rate of oxidation, which is inversely related to the rate of sedimentation²². Similar oxidation-reduction behaviour has been proposed to explain the relative distributions of chromium¹⁴ and of uranium²³ in the Bauer Basin and East Pacific Rise sediments.

We plan further work on submarine mercury distributions, and are currently investigating sediments from back-arc spreading centres and marine manganese deposits. On the Fiji Plateau, where mercury concentrations in surface sediments are

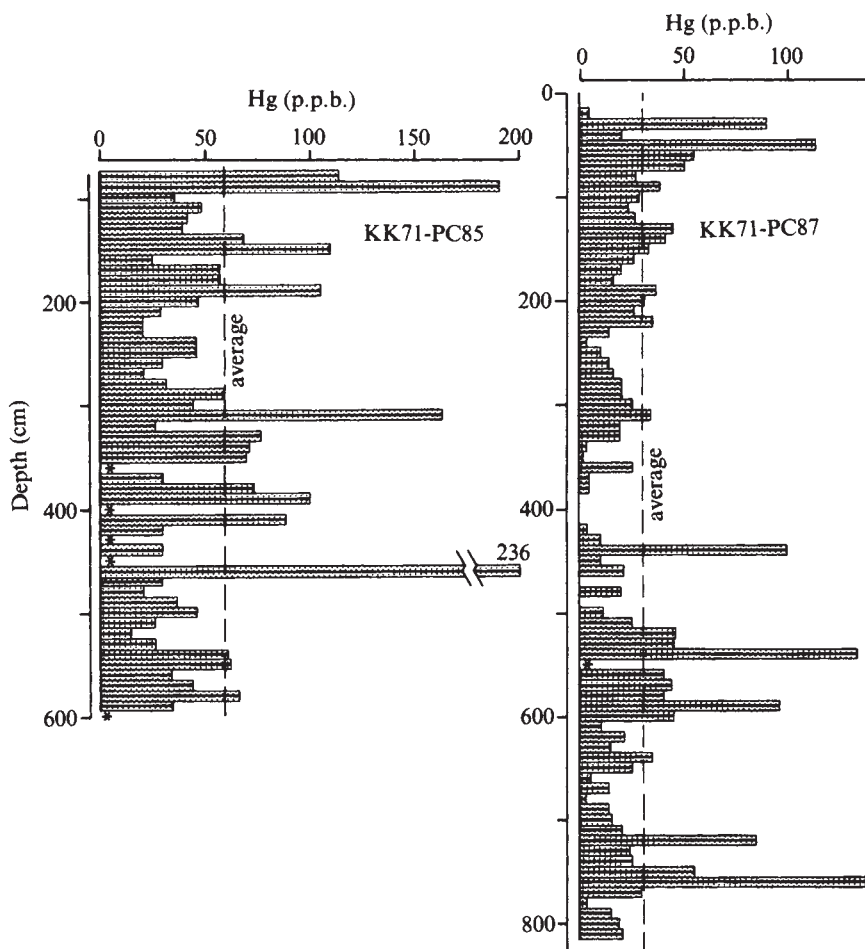


Fig. 2 Mercury concentration plotted against depth for the East Pacific Rise sediment cores. Because of coring disturbances, the topmost 70 cm of core KK71-PC85 was not analysed. Bar width indicates vertical uncertainty ($\pm 5 \text{ cm}$) due to sampling interval. The vertical width of each sample for mercury analysis was within $\sim 1 \text{ cm}$. * Not determined.

generally low (<10–70 p.p.b.), the highest values correlate with increased heat flow²⁴. Concentrations of mercury are extremely variable within marine manganese nodules (<1–810 p.p.b.), but are highest (>58 p.p.b.) in nodules from sea floor areas characterized by volcanic and hydrothermal features²⁵. Similarly, mercury concentrations from several hundred to >2,000 p.p.b. were measured in ferromanganese crusts from active spreading centres²⁶.

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Precambrian microfossils from the Sinian of South China

Zhang Zhongying*

Department of Geology, Nanjing University, Nanjing, China

The discovery of previously unknown microfossils from the Sinian of western Hubei Province, South China (Fig. 1) is reported here. The Sinian there, up to 1,000 m thick, is naturally divisible into four formations (Fig. 2) from the base upwards: (1) the Liantuo Formation comprising feldspar-quartz-sandstones with basal conglomerates; (2) the Nantuo Formation, which is a tillite; (3) the Doushantuo Formation, which has micro-dolomites intercalated with black shales and cherty beds with some siliceous or phosphatic nodules; and (4) the Dengying Formation, which is mainly dolomites. The rock sample in which the microfossils were found was collected from the lower part of the Doushantuo Formation near the highway side ~500 m east of Liantuo, Yichang County (Fig. 1). The Doushantuo Formation has been dated as 693±66 Myr (Rb-Sr isochron age)¹. Pseudomorphs after gypsum sometimes occur in the lower part. The microfossils are preserved in siliceous nodules, particularly in the cherty portions. The dark colour of the nodules is due to the high organic content.

These new Doushantuo microfossils were discovered in petrographical thin sections and include at least four types—spheroids, colonies composed of unicells, nonseptate filaments

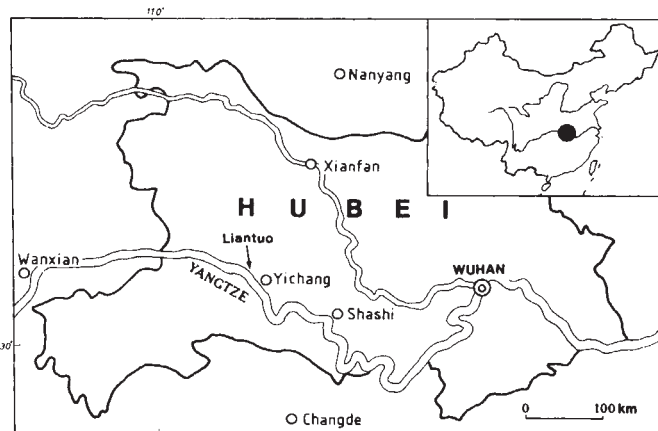


Fig. 1 Locality map.

and septate filaments. In some, fine structural details are well preserved, while others are modified or destroyed by diagenetic and subsequent processes. Figure 3 shows only a few of the new Doushantuo assemblage and a more detailed systematic account will be published elsewhere.

Spheroidal members (Fig. 3b–d) of the assemblage range from ~8 to 18 µm in diameter, and exhibit granular or psilate surface textures. Although mainly solitary, and not associated with other organic matter, they are occasionally paired, or grouped in clusters. Some seem to contain inner dark spots ~2 µm across (Fig. 3c). In many colonies (Fig. 3h) where single spheroids 2–9 µm in diameter occur associated spheroids have also been found. Colonies may be up to >100 µm across, comprising a few to several tens of cells which set in a matrix of apparently mucilaginous material. Some of these colonies seem to have been disintegrated into smaller ones or into separate cells. These spheroids and colonies composed of unicells might represent chroococcacean cyanophytes, although some have uncertain affinities.

Both non-septate and septate filaments occur in the Doushantuo Formation, and some probable isolated trichomes and slime trails left by gliding filamentous algae or bacteria can be found. Organic walled non-septate filaments (Fig. 3 e–g) are generally straight or slightly sinuous, unbranched, 2–9 µm in diameter. Their maximum overall length is 56 µm. Occasionally loosely spirally twisted filaments have been found in petrographical thin sections. Variations in preservation may give these filaments a superficially septate appearance (Fig. 3g). Most of the non-septate filaments are interpreted to be discarded sheaths that originally enclosed cellular blue-green algal

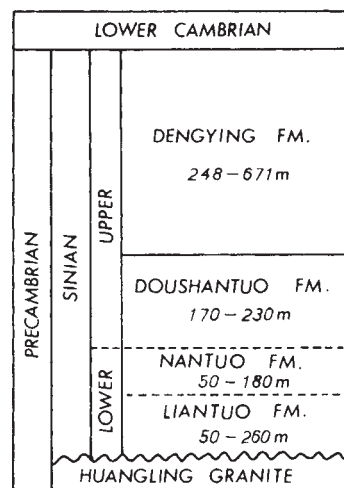


Fig. 2 Generalized stratigraphy of the Sinian in western Hubei Province, China.

* Present address: Department of Geology, Royal School of Mines, Imperial College, London SW7 2BP, UK.

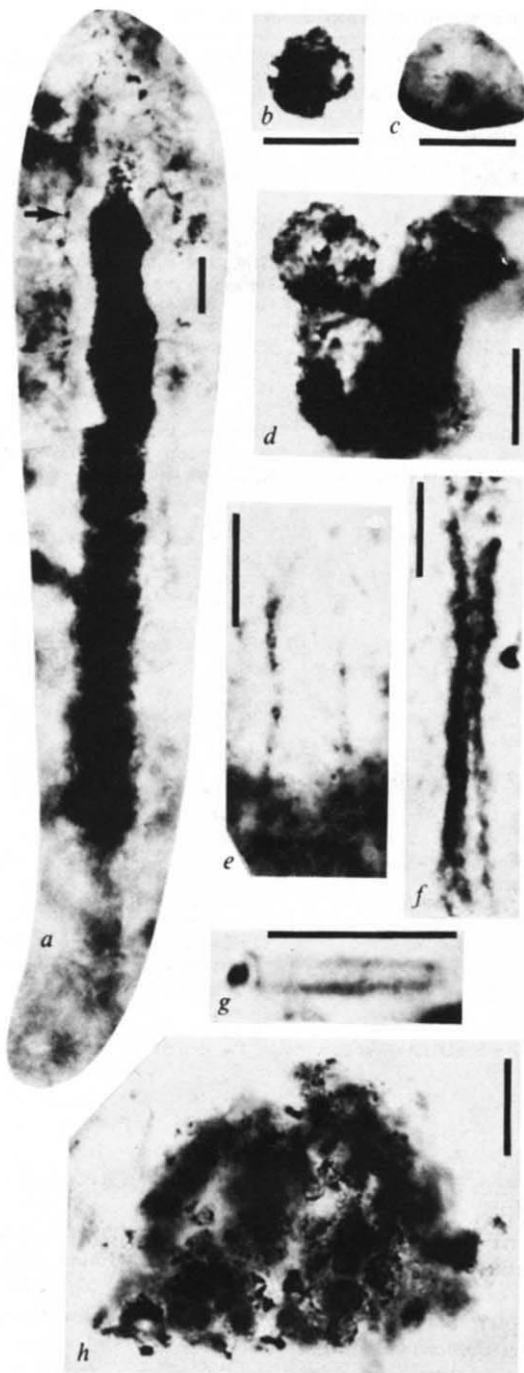


Fig. 3 Organically preserved microfossils from the Doushantuo Formation (late Sinian) of western Hubei Province, China. All micrographs are of petrographic thin sections in plane polarized light. *a*, A photomontage. Scale bars, 10 μm .

trichomes, while the narrower ones probably represent filamentous bacteria or oscillatoriacean algae.

Although septate filaments are rare and occur in a poor state of preservation, one nearly complete specimen (Fig. 3*a*) has been found. The filament is solitary and has a straight to slightly curved shape. The trichome composed of disk-shaped cells 9.0–11.0 μm in diameter and 3.5–5.5 μm in length is uniseriate, unbranched, not constricted or slightly constricted at septa and gradually tapering towards apices. The sheath (Fig. 3*a*, arrow) is hyaline, nonlamellated and up to 7.5 μm thick, gradually tapering towards apical portion. This specimen seems comparable with oscillatoriacean blue-green algae. Oscillatoriaceae-like filaments are very common and important among Precambrian

microfossils, which have been found throughout the world^{2–7}. The present form constitutes the first reliable record of them in the Precambrian rocks of China.

Sedimentological studies indicate that the Doushantuo Formation represents shallow water marine sediments formed in an arid climate¹. The occurrence of the new Doushantuo assemblage predominantly composed of coccooid and filamentous cyanophycean algae supports this view. These microfossils seem comparable with some stromatolite-building organisms found in many Precambrian formations^{2,5,8–11}. However, in China although rich microfossil assemblages and stromatolites have been described from Precambrian rocks, our knowledge about stromatolite-building organisms in these rocks remains very meagre, and few stromatolites from the Doushantuo Formation of South China have been reported¹². On the other hand, this newly discovered microbiota represents one of the youngest Precambrian microfossil assemblages now known; others include those from the Vendian of South Kazakhstan^{10,13}, the Arabian Shield¹⁴ and the Lower Yudoma Suite¹⁵.

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Asymmetrical competition in insects

J. H. Lawton

Department of Biology, University of York, Heslington, York YO1 5DD, UK

M. P. Hassell

Imperial College at Silwood Park, Ascot, Berks SL5 7PY, UK

It is widely believed that when populations of two species of animals compete, each has an adverse effect on the other^{1,2}. In other words, each zero growth isocline ($dN_i/dt = 0$ where N_i is the population density of species i) is assumed to be some negative function of the population density of the other species. Cases where one species has a marked effect on the other, but there is no detectable reciprocal effect are sometimes distinguished as 'amensalism'^{2,3}. This is regarded as unusual. Standard textbooks of ecology either do not mention it^{1,4–6}, define it but do not discuss it², or occasionally give a brief account³. Ricklefs⁷ states that "competition can manifest itself by reducing the numbers of one or both competing species" without saying which is the more usual. Here we show that for insects in natural conditions, strongly asymmetrical competition (amensalism or near amensalism) is the norm rather than the exception by a ratio of at least 2:1.

Table 1 Examples of amensalism (strongly asymmetrical competition) between insect populations in the field

Ref.	Dominant (<i>j</i>)	Subordinate (<i>i</i>)	Mechanism	Taxonomic group, habitat and food
26	<i>Calliphora auger</i>	<i>Lucilia cuprina</i>	Resource exploitation and possible habitat modification	Diptera: sheep carrion
26	<i>Chrysomya rufifacies</i>	<i>Lucilia cuprina</i>	Resource exploitation and aggression	Diptera: sheep carrion
28	'Early' Odonata	'Late' Odonata	Predation	Odonata: aquatic predators
30*	<i>Danaus plexippus</i>	<i>Oncopeltus</i> spp.	Extreme exploitation of food-plant	Lepidoptera and Hemiptera: milkweed plants
23	<i>Bombus pratorum</i>	<i>Bombus agrorum</i>	Aggression	Hymenoptera: artificial, experimental flowers
27†	<i>Sigara macropala</i>	<i>Hesperocorixa lobata</i>	Exploitation?	Hemiptera: aquatic detritivores
40	<i>Dacus dorsalis</i>	<i>Ceratitis capitata</i>	Larval interactions; mechanism?	Diptera: guave fruits
22	<i>Prenolepis imparis</i>	<i>Aphaenogaster rudis</i>	Aggression	Hymenoptera: experimental protein baits in woodland
41*	Several ant species	<i>Macromischoides aculeatus</i>	Aggression?	Hymenoptera: predators in cocoa plantations
42	<i>Fiorinia externa</i>	<i>Tsugaspidotus tsugae</i>	Resource exploitation and action of shared parasitoid	Homoptera: eastern hemlock foliage
29	<i>Cromaphis juglandicola</i>	<i>Panaphis juglandis</i>	Habitat modification via excretion	Homoptera: walnut foliage
24	<i>Bombus terricola</i>	<i>Bombus ternarius</i>	Behavioural avoidance by subordinate	Hymenoptera: goldenrod flowers
25	'Mordellidae 23'	<i>Epiblema</i> sp.	Aggression and death of subordinate	Coleoptera and Lepidoptera: plant stems in prairie
43	<i>Copestylum</i> cf. <i>obscurior</i>	<i>Quichuana picadoi</i>	?	Diptera: water-filled <i>Heliconia</i> bracts: nectar, detritus and plant tissue
43	<i>Gillisia</i> sp.	<i>Beebeomyia</i> sp.	?	Coleoptera and Diptera: water-filled <i>Heliconia</i> bracts: plant tissue
43	<i>Gillisia</i> sp.	<i>Cephaloleia puncticollis</i>	?	Coleoptera: water-filled <i>Heliconia</i> bracts: plant tissue
43	<i>Merosargus</i> sp.	<i>Gillisia</i> sp.	?	Diptera and Coleoptera: water-filled <i>Heliconia</i> bracts: detritus and plant tissue
43†	<i>Quichuana picadoi</i>	<i>Gillisia</i> sp.	?	Diptera and Coleoptera: water-filled <i>Heliconia</i> bracts: detritus and plant tissue
44	<i>Copestylum roraima</i>	<i>Cephaloleia neglecta</i>	Disturbance and behavioural avoidance	Diptera and Coleoptera: water-filled <i>Heliconia</i> bracts: detritus and plant tissue
45	<i>Cephaloleia neglecta</i>	<i>Xenarescus monocerus</i>	Interference leading to emigration	Coleoptera: <i>Heliconia</i> bracts
18	<i>Urophora solstitialis</i>	<i>Rhinocyllus conicus</i>	Habitat modification via gall formation	Diptera and Coleoptera: plant tissue in flowerheads of thistle
18	<i>Larinus sturnus</i>	<i>Rhinocyllus conicus</i>	Probably exploitation of resources	Coleoptera: plant tissue in flowerheads of thistle
18	<i>Encosma</i> and <i>Homoeosomo</i>	All other species in flower heads	Aggression and predation	Lepidoptera and other: plant tissue in flowerheads of thistle

The dominant species, *j*, has a marked effect on the subordinate, *i*, ($\alpha_{ij} > 0$) whilst the reciprocal effect is negligible ($\alpha_{ji} \approx 0$). Less certain, probably amensal, examples are marked *. There are two cases (†) in which the inferior species (*i*) seems to have a beneficial effect on *j*, moving the zero growth isocline of the latter clockwise, to intersect the N_1 axis of Fig. 1b at $>90^\circ$.

The view that interspecific competition plays a key part in structuring guilds of birds and mammals is central to current thinking in community ecology^{8,9}. Insects have been relatively neglected in this context, despite being the most important terrestrial, and amongst the most important freshwater, consumers. Insects have vastly more species¹⁰ and frequently dominate terrestrial communities in terms of biomass and energy transfer. Widespread amensalism amongst insects would thus have important implications for theoretical community ecology.

Having reviewed the evidence for interspecific competition amongst insect populations in the field¹¹, we have found major and consistent differences between groups in the apparent importance of competition. Thus, whilst the ants and bees provide many good examples of competition, competition for food between phytophagous species is unusual¹². Where species do compete, irrespective of their taxonomic group or the type of resource exploited, competition is generally highly asymmetrical. The 23 such examples that we have found are listed in Table 1; they probably provide a conservative total as some involve more than one pair of species. Only those cases where we can be reasonably certain of the degree of asymmetry in the interaction have been included. There are several other reports in which the effects of one species on another are clearly established, but the magnitude of the reciprocal interactions is not given, or cannot be deduced with any degree of certainty (see refs 13, 14 for example). Note that the criterion for amensalism is not simply that one species excludes another: this may happen

when both α_{ij} and $\alpha_{ji} > 0$. Instead we require that $\alpha_{ij} > 0$ but $\alpha_{ji} \approx 0$ (Fig. 1).

We have found only five reports of unequivocal, reciprocal competition involving insect populations in the field—four species-pairs and one larger group. Two species of leaf-feeding Homoptera in the genus *Eupteryx* compete reciprocally¹⁵, as do the bees *Bombus appositus* and *Bombus flavifrons*¹⁶, the aquatic Hemiptera *Arctocoris carinata* and *Callicorixa producta*¹⁷, and a pair of parasitic Hymenoptera in the genus *Eurytoma*¹⁸. In addition, McClure and Price¹⁹ demonstrate competition in eight pair-wise experiments involving seven species of Homoptera all in the genus *Erythroneura*. If all eight pairs of interactions show statistically significant reciprocal effects (it is not clear from their data whether this is so), the ratio of examples showing amensalism (Table 1) to those showing more symmetrical competition is approximately 2:1 (23:12). If we choose to count the intensely studied genus *Erythroneura* only once, this ratio rises to nearly 5:1 (23:5). Whatever the exact ratio, the notion that amensalism is unusual is clearly not supported by the data: quite the reverse is true.

Obviously, the data must be interpreted carefully. We have probably overlooked some examples and may have misinterpreted others. Omission of uncertain but possibly symmetrical examples is balanced by cases where amensalism is probable but not proved²⁰. Similarly, although there are cases of partial behavioural dominance, and hence possible reciprocal competition (for example, the ants *Pogonomyrmex rugosus* and *Pogonomyrmex desertorum*²¹), these are balanced by examples

of almost certain total dominance (*Camponotus* ants frequently kill, maim and displace *Aphaenogaster* in confrontation at food sources²²). Complete exclusion from preferred resources²²⁻²⁴ undoubtedly affects colony dynamics in a highly asymmetrical manner. In brief, we think it unlikely that enough examples have been overlooked or misinterpreted to alter our conclusions significantly.

Given the difficulties in determining levels of competition in field conditions and the inevitable noise in the data, it is, of course, impossible to distinguish cases of true amensalism ($\alpha_{ij} > 0$; $\alpha_{ji} = 0$) from cases which are nearly so ($\alpha_{ji} \approx 0$), and there is undoubtedly a continuum of examples from the truly symmetrical ($\alpha_{ij} \approx \alpha_{ji} > 0$) to complete amensalism. However, the evidence suggests that examples are not uniformly distributed along this continuum. Strongly asymmetrical competition seems to be the general case, rather than the exception.

The examples of strongly asymmetrical competition summarized in Table 1 cover a wide range of feeding behaviour, including phytophages²⁵, carrion feeders²⁶, aquatic detritivores²⁷, predators²⁸ and nectivores²⁴. They also arise in several very different ways, involving simple avoidance behaviour²⁴ of one species by another, direct aggression²²

(including, in extreme cases, the death of the inferior competitor²⁵), habitat modification²⁹ or simple exploitation of resources³⁰. In several cases the mechanism is not known with certainty; only its effect. The breadth of these examples, and the range of habitats and taxa involved, strengthen our belief that strongly asymmetrical competition is the norm in insects.

Strongly asymmetrical competition approaching amensalism has been widely reported in other groups of animals and, just as for insects, may well be much commoner in these than has generally been acknowledged. Examples include organisms as different as barnacles³¹, hummingbirds³², small mammals³³ and wildebeest and buffalo³⁴. Morse³⁵ provides an extensive review of social dominance between animal species (mainly mammals, birds and fish, but also nine insect examples and one involving Crustacea). Interactions are usually high asymmetrical, particularly where the fundamental niche of an aggressively dominant species is 'included' within that of a subordinate species^{35,36}. We emphasize, however, that our examples include several forms of competition, not just social dominance.

We expect the dynamics of species associations in which amensalism is the norm to be very different from those dominated by symmetrical interactions. For example, predictions about niche shifts³², stability^{37,38} and estimates of connectance³⁹ are all likely to be affected. We believe that studies of community organization for those groups of species in which competition is known to be important should now pay much more attention to highly asymmetrical interactions, treating amensalism as a common, widespread form of species interaction.

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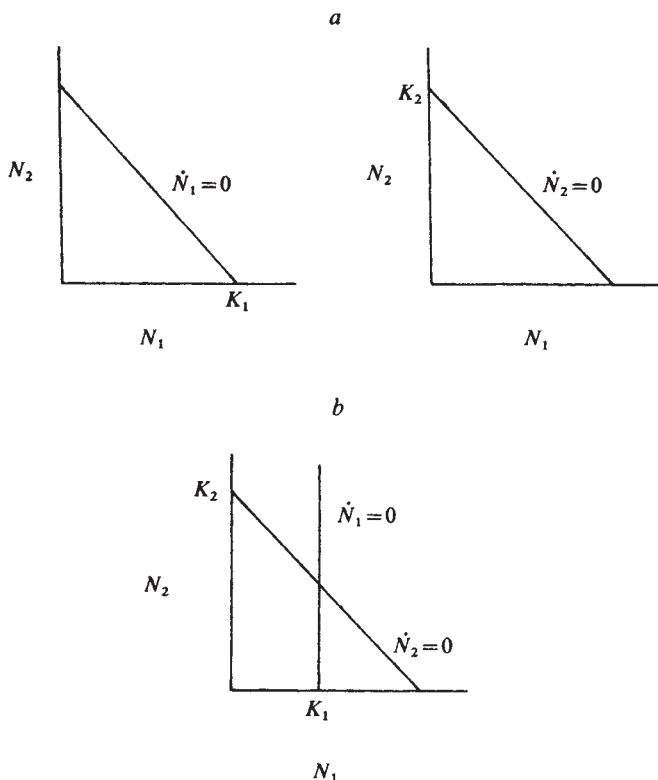


Fig. 1 a, Zero growth isoclines for the conventional two-species Lotka-Volterra competition model¹⁻³.

$$\frac{dN_1}{dt} = r_1 N_1 (1 - N_1/K_1 - \alpha_{12} N_2/K_1)$$

$$\frac{dN_2}{dt} = r_2 N_2 (1 - N_2/K_2 - \alpha_{21} N_1/K_2)$$

N refers to the population densities of species 1 and 2, r to their intrinsic rates of increase, K to their carrying capacities, and α_{12} and α_{21} , the competition coefficients, to the competitive effects of one species on the other. Stable coexistence or the exclusion of one species by another depends on the relative position of the two isoclines. b, With amensalism, the population of one species (species 2 in this example) is markedly affected by the other (that is, $\alpha_{21} > 0$), but the reciprocal effect is zero ($\alpha_{12} = 0$). If the isoclines intersect, as shown here, both species coexist. Otherwise the affected species (species 2) is inevitably excluded.

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Time course of *O*⁶-methylguanine removal from DNA of *N*-methyl-*N*-nitrosourea-treated human fibroblasts

A. S. C. Medcalf* & P. D. Lawley

Pollards Wood Research Station, Institute of Cancer Research,
Nightingales Lane, Chalfont St Giles,
Buckinghamshire HP8 4SP, UK

Methylating mutagens are thought to act by induction of direct miscoding, mainly through formation of *O*⁶-methylguanine (*O*⁶MeG) in DNA of, for example, T-even bacteriophage¹, *Escherichia coli*², and a cultured Chinese hamster cell line³. A positive correlation between this reaction in DNA of target tissues and subsequent induction of tumours has been found for rat kidney tumours⁴ and thymic lymphomas in mice⁵. These effects are thought to be counteracted by an enzymatic repair process, which removes *O*⁶MeG from DNA (first found in *E. coli*⁶, then in liver of rats⁷ and mice⁸). The predominance of liver as the site of this action *in vivo* led to suggestions⁷ that this could account for the relative lack of susceptibility of liver tissue to methylating carcinogens, and also that the enzyme involved might be inducible⁸. Evidence for the latter has been claimed for rat liver⁹, but is most convincing in *E. coli*¹⁰ where inhibition of this enzymatic action is achieved by treating cells with chloramphenicol before methylation¹¹, thus specifically inhibiting protein synthesis. As there is very limited removal of *O*⁶MeG from methylated cellular DNA, compared with that of 3-methyladenine (3-MeA, a suggested DNA template-inactivating base), it is thought that the former reaction is mediated by a protein which is consumed by the reaction¹². There have been reports^{13,14} that human cells also can remove *O*⁶MeG, but the data did not clarify the time- and dose-dependencies of the process. We report here more effective removal at lower extents of methylation, which suggests a nonlinear dose response to methylating mutagens and carcinogens for human cells. There were no significant deficiencies in methylpurine removal from DNA of patients with ataxia telangiectasia (AT) or xeroderma pigmentosum (XP).

We used untransformed rather than transformed cells as the latter might be relatively deficient in DNA repair capability; density-inhibited cultures¹⁵ were used as it was considered necessary to treat the different cell strains in identical conditions. This eliminated possible cell cycle effects and also DNA synthesis as complicating factors. The cells used were 1BR2, 2BI (radiation repair-proficient), AT3BI and XP12BE (radiation repair-deficient).

Human fibroblasts were treated with the methylating carcinogen *N*-[¹⁴C]methyl-*N*-nitrosourea (MNUA) at doses of ~0.1 mM and ~0.5 mM and the amounts of ¹⁴C-methylated purines in cellular DNA were determined after various times of incubation. 3-MeA was rapidly removed from DNA at both doses with a half life of ~2 h; the half life of 7-MeG was ~30 h. These rates are much greater than for the spontaneous hydrolytic loss of these purines from DNA and therefore indicate enzymatic removal processes. The chemically stable *O*⁶MeG was also removed from DNA (possibly by demethylation as in *E. coli*¹⁶) but the kinetics differed from those for the other methylpurines—a rapid component was found early on which removed ~2–4 μmol *O*⁶MeG per mol DNA-P (~4–8 × 10⁴ molecules per cell) in the first 1–2 h, then the rate decreased to about one-fifth of its initial value. At the lower doses of MNUA, 90% of the *O*⁶MeG was removed within 10 h, whereas at the higher

dose level over half still remained. Regarding the hypothesis that *O*⁶MeG is a directly miscoding base, which causes mutation and tumour initiation if not removed before replication of methylated DNA templates, more effective removal at lower extents of methylation suggests a nonlinear dose response.

The results are summarized in Fig. 1 and Table 1, presented respectively as amounts of methylated bases per 10⁶ DNA nucleotides, and as ratios of methylated bases at various incubation times after methylation. No corrections were required for dilution of bases by DNA synthesis, as there was no significant reduction of specific radioactivity of ³H-thymidine pre-label in cellular DNA. Clearly the methylpurines examined were actively removed from DNA, 3-MeA and 7-MeG much more rapidly than occurs by spontaneous hydrolysis (half lives of ~30 and ~110 h respectively)¹⁷.

Figure 1 shows that the time course of removal of each methylpurine from DNA had its own specific characteristics. 7-MeG declined with approximately first-order kinetics (linear semi-logarithmic plot) and with the same half life at both dose levels. 3-MeA was removed much more rapidly, with nonlinear kinetics, but again the efficiency of removal was independent of dose. However, *O*⁶MeG showed markedly nonlinear kinetics, and approximated to a two-component time course, with a higher efficiency of removal at the lower dose than at the higher.

This conclusion rests on the assumption that the initial extents of methylation of cellular DNA at the O-6 atom of guanine can be calculated as 0.11 times the extent of methylation at the N-7 atom, that is, they are identical to those found for methylation of DNA in the isolated state *in vitro*, or in cells which cannot remove this base³. These calculated values are indicated by arrows on the y axes of Fig. 1. The extrapolations of the approximately linear portions of the graphs for the higher doses (Figs 1b, d) to zero time are quite close to, but significantly lower than, these calculated values. The earliest time points for which analyses of DNA could be carried out were 1–2 h after addition of the methylating agent, to allow for completion of the methylation reaction (half life ~0.2 h) and collection of cells for DNA isolation.

During this time, as is evident from the time courses for removal of *O*⁶MeG (Fig. 1) ~2 μmol per mol DNA-P atoms (or DNA nucleotide units) were removed at the lower dose, and ~3–4 μmol per mol DNA-P at the higher dose—this represents a much higher proportion of the initial reaction at the lower (~3 μmol per mol DNA-P) than at the higher dose (15–20 μmol per mol DNA-P). It was calculated from the known DNA content of the cells (~2 × 10¹⁰ DNA-P) that this rapid initial component removed 4–8 × 10⁴ molecules *O*⁶MeG per cell per h, or ~1,000 per cell per min.

The rate of removal then declined at both dose levels, the linear portion of the graphs at the higher dose showing a rate of ~200 molecules per cell per min. These kinetic characteristics could be considered to support the view¹² that the initial process is 'quasi-enzymic', that is, that the protein involved is consumed by the reaction, and is initially present in a limited amount.

In view of reports that in *E. coli* this enzyme (or quasi-enzyme) is inducible by pretreatment of cells with low doses of methylating agents¹², the question arises of whether the enzyme in human cells is similarly inducible. Evidence for this is not yet available, but the observed rapidity of the initial component reaction in the present work contraindicates its induction by the experimental treatment. This follows from the finding that removal of *O*⁶MeG can be at least as rapid in human cells as that of 3-MeA, whereas in *E. coli* removal of the latter, which is effected by a constitutive enzyme, is more rapid, and enzyme induction in mammalian cells is expected to be a much slower process than in bacteria¹⁸. It is possible that, due to low levels of methylating carcinogens in the diet (for example, dimethyl-nitrosamine in meat products¹⁹), the enzymatic activity in isolated cells could have been preinduced in the donors. It might be difficult to distinguish between such preinduced activity and constitutive activity, but the persistence of preinduced activity through many cell generations in culture seems improbable.

* Present address: Carcinogenesis Laboratory, Fee Hall, Michigan State University, East Lansing, Michigan 48824, USA.

Table 1 Ratios of methylated bases at various incubation times after methylation

Cell line	MNUA dose (mM)	Time (h)	% Survival	O ⁶ -MeG/7-MeG	3-MeA/7-MeG
1BR2	0.11	1.2	90.0	0.036	0.061
		5.5	—	0.011	0.005
	0.46	1.2	14.8	0.077	0.053
		4.0	—	0.070	0.015
		23.0	50.5	0.079	—
AT3BI	0.09	1.2	—	0.050	0.044
		4.0	—	0.019	0.035
		20.0	—	0.005	0.005
	0.50	1.0	1	0.090	0.049
		4.0	—	0.091	0.022
		21.0	38.3	0.090	0.008
2BI	0.18	1.8	—	0.035	0.061
	0.51	1.1	13.0	0.082	0.058
		2.8	—	0.089	0.031
		24.0	58.7	0.051	—
XP12BE	0.10	1.2	—	0.032	0.050
		28.0	—	0.006	0.018

Values of extents of methylation of DNA were obtained as described in the legend to Fig. 1, and additional experiments have confirmed the results with 1BR2, AT3BI and XP12BE. Survivals were determined by collecting density-inhibited cultures by trypsinization, and plating appropriate numbers of cells to allow for colony growth at plating efficiencies of 10–40%, using feeder layers as described elsewhere²⁴. Survival is colony-forming ability of treated cells divided by that of controls, only colonies with >50 cells being scored. Additional experiments (data not shown) were done to show that survival in all cases increased to almost 100% after ~40 h incubation in the density-inhibited state.

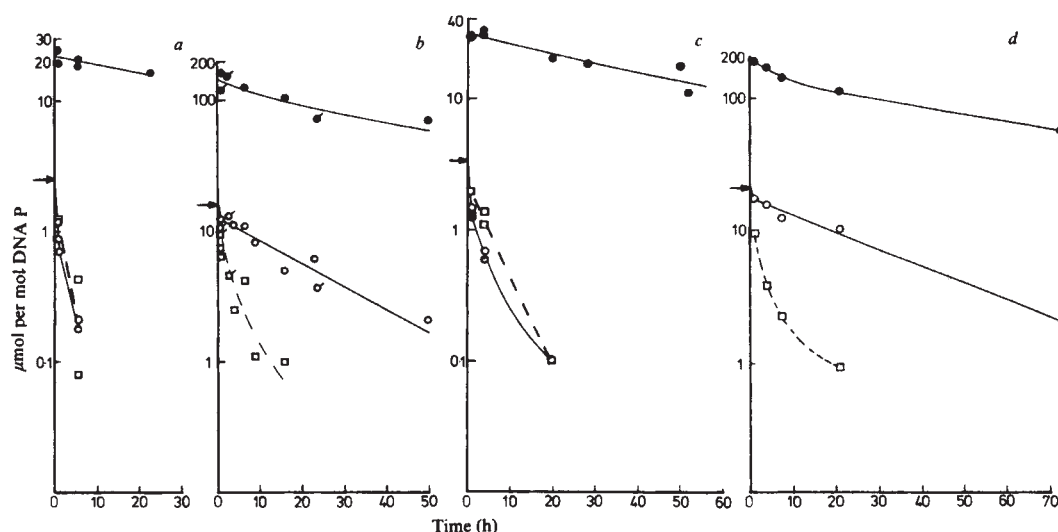


Fig. 1 These experiments were carried out in as near similar conditions as possible to eliminate the effects of variables such as DNA synthesis. The use of confluent cells was considered the most appropriate and convenient way of achieving this. Cells were obtained in this condition by a modification of the method of Weichselbaum *et al.*¹⁵. Cells (2×10^6) were inoculated into plastic tissue culture flasks (Nunc, 175 cm²) containing Dulbecco's modified minimal essential medium (50 ml) supplemented with 15% fetal calf serum (Gibco). After incubation at 37 °C (48 h), ³H-thymidine (Radiochemical Centre) was added ($3 \mu\text{Ci mmol}^{-1}$, 7.4 μM), and the cells were collected after 24 h using trypsin/EDTA, pooled and the required number of flasks seeded with 2×10^6 cells in 50 ml medium; this ensures that there is equal specific radioactivity of ³H-thymidine in each flask. There were only negligible decreases in the specific radioactivities of ³H-thymidine in DNA over the experimental period, as expected for density-inhibited cells. The flasks were further incubated at 37 °C until confluence was reached, when the medium was collected for use as conditioned medium. This was repeated twice at daily intervals and the experiment was carried out 24 h after the last re-feed. Medium was removed from each flask, and the cells were washed once in prewarmed phosphate-buffered saline (PBS), residual PBS being removed by pipette. ¹⁴C-MNUA (Radiochemical Centre, 37 mCi mmol⁻¹) was added to prewarmed PBS to give the required concentration for treatment, from a 1:200 volume of a stock solution in anhydrous dimethyl sulphoxide (DMSO), and 10 ml was added to each flask. The concentration was checked by assay of the radioactivity; controls were treated with DMSO (0.5% v/v). This concentration of DMSO was non-toxic and experiments with 1% (v/v) or no DMSO gave identical results. Treatment was for 40 min at 37 °C in the dark and was terminated by removal of radioactive PBS, and washing the cells once with warm PBS. At this stage and at appropriate times after incubation in conditioned medium, cells were collected from 1–4 flasks (dependent on the dose used). Sufficient cells were retained for assay of survival by the method of Cox and Masson²⁴. The remaining cell residue ($0.3\text{--}1.5 \times 10^8$ cells) was used for isolation of DNA by a modification of the method of Kirby²⁵. DNA (0.2–1 mg) was hydrolysed in 0.1 M HCl at 70 °C to liberate purine bases and analysed chromatographically using HPLC on a Partisil 10-SCX column eluted with a gradient of 0.05–0.4 M ammonium formate, 12–10% methanol, essentially as described by Frei *et al.*⁵ Guanine and adenine were determined in each chromatogram by UV absorption of the appropriate fractions: ϵ_{max} (guanine) 8,000 at 273 nm; ϵ_{max} (adenine) 13,100 at 260 nm. The amount of DNA-P was then calculated as $2 \times (\text{mol guanine} + \text{mol adenine})$. This gave the extents of methylation of DNA, principally at O-6 and N-7 of guanine and N-3 of adenine. The initial extents of reaction at O-6 of guanine were calculated as 0.11 times the values for 7-MeG and are shown by arrows. a, 1BR2 treated with MNUA, (~0.1 mM); b, 1BR2 and 2BI (denoted by $\circ$, $\square$ and $\bullet$) treated with MNUA (~0.5 mM); c, AT3BI treated with MNUA (~0.1 mM); d, AT3BI treated with MNUA (~0.5 mM). $\circ$, O⁶-MeG; $\square$, 3-MeA; $\bullet$, 7-MeG.

However, the slower component of the removal could be induced by the methylation damage to DNA.

Regarding a possible association between inherited defects in repair capability for radiation-induced damage in DNA and defective ability to remove methylpurines, our results showed no evidence for such an association in the AT or XP cells used. Previous reports^{13,14} which claimed XP-associated defective repair of methylation damage were based on the use of virally transformed XP cells; therefore it cannot be ruled out that the defective removal of O^6 -MeG was associated with the transformed nature of the cells, and not with any defect in UV repair capability.

Several implications for consideration of dose-response relationships in the associated mutagenic, transforming and tumour initiating effects of methylating carcinogens can be made from our results. Such relationships could explain why human cells have proved refractory to malignant transformation *in vitro* by such agents. One study²⁰ concluded that this transformation, by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine required a relatively high degree of toxic action of the methylating agent, killing about 70% of the cells, that is, a correspondingly high extent of methylation of cellular DNA, possibly sufficient to reduce markedly the efficiency of O^6 -MeG removal.

The levels of methylating carcinogens in the human diet are probably low¹⁹ (for example, ~1–10 parts per 10⁹ of dimethylnitrosamine (DMNA) was estimated in certain meat products) and it seems likely that these would lead to sufficiently low extents of DNA methylation to be removed very efficiently in cells with the same ability to carry out this reaction as the fibroblasts of the present study.

Analogous ability of cells of rat liver to remove this miscoding base from DNA, methylated by continuous feeding with DMNA, has been suggested⁷ to account for the relatively low (or, on the concept that a no-effect threshold dose exists, zero) yields of induced liver cell cancer at lower dose levels (suggested threshold of 0.1 parts per 10⁶)²¹.

It is also relevant to consider the frequency of UV-induced mutation in human fibroblasts, which shows a no-effect threshold²². This threshold is associated both with efficient repair of UV-induced DNA damage, and with protection against the induction of mutation and cancer, because XP cells, deficient in this repair, show no threshold, and are more prone to the mutagenic and carcinogenic effect of UV light.

Another case of a markedly nonlinear dose response has been reported²³ for induction of mutation in human lymphoblasts by a methylating carcinogen, *N*-methyl-*N*-nitrosourea, and this could also be ascribed to more efficient removal of the promutagenic base O^6 -MeG at low doses.

Taken together, these considerations lead to the general hypothesis that cells with the ability to remove O^6 -MeG from DNA more efficiently at lower extents of methylation, will show a markedly nonlinear dose response for malignant transformation by methylating carcinogens, with a very low probability per unit dose at low doses. In the present study the human cells examined were in this category and therefore should be correspondingly resistant to low doses of methylating carcinogens.

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Distinct lung-colonizing and lung-metastasizing cell populations in B16 mouse melanoma

Christopher W. Stackpole*

Memorial Sloan-Kettering Cancer Center, Rye, New York 10580, USA

Establishment of distant metastases by solid tumours requires that some tumour cells separate from the primary growth, infiltrate surrounding normal tissues, invade blood vessels or lymphatic channels, and extravasate into microenvironments favourable for proliferation¹. It is probable that only a small subpopulation of tumour cells is endowed with these special attributes². The biological properties of potentially metastatic tumour cells are being investigated extensively by means of an 'experimental metastasis' assay, in which cultured cells are introduced directly into blood vessels and, several weeks later, organs examined for tumour growths^{1–4}. Although this assay allows controlled and quantitative experimentation, the suitability of experimental metastasis (colonization) as a valid representation of true metastasis is uncertain. I report here that organ colonization after injection of cells directly into the blood vasculature is not necessarily predictive of spontaneous metastatic potential from subcutaneous transplants. Indeed, some cell lines derived from the B16 mouse melanoma produce numerous lung colonies when injected intravenously (i.v.) but rarely metastasize, whereas others are spontaneously metastatic to the lungs but produce few lung colonies after i.v. injection. These differences in behaviour are evident only when the possibility of colonization occurring due to accidental introduction of melanoma cells into blood vessels is excluded.

B16 melanoma cell lines were maintained as monolayer cultures in 75 cm² plastic tissue culture flasks with Eagle's minimal essential medium containing 10% fetal bovine serum, 1.5% vitamins, 1% nonessential amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine, 100 units ml⁻¹ penicillin, and 100 µg ml⁻¹ streptomycin, pH 7.0. Primary lines were established from single-cell suspensions prepared from subcutaneous (s.c.) or intraperitoneal (i.p.) tumour transplants, lung colonies, or lung metastases, by mincing the tissue with scissors and pressing it through 80-mesh stainless-steel wire screens, and washing by low-speed centrifugation, in sterile conditions. Before experiments, monolayers were initiated from frozen stocks by seeding 5 × 10⁴ viable cells and were collected ~24 h before confluence by incubation for 1 min at 37 °C with 1 ml of Hank's balanced salt solution, without calcium or magnesium, supplemented with 0.25% trypsin, 0.02% EDTA and penicillin-streptomycin. Single-cell suspensions free of cell clumps and >95% viable were washed twice with Earle's balanced salt solution containing 1% heparin and penicillin-streptomycin

* Present address: Department of Medicine, New York Medical College, Valhalla, New York 10595, USA.

Table 1 Lung colonization by B16-F10 cells injected at various anatomical sites

Injection site	No. of mice with lung colonies	Median no. of lung colonies
Lateral tail vein (i.v.)	20/20	88 (28–250)
Hind leg, footpad (i.m.)	7/20	6 (1–14)
External ear (i.d.)	3/20	12 (9–16)
Hind leg, thigh (s.c.)	5/20	5 (2–10)
Abdominal flank (s.c.)	0/20	0

Female mice 8–10 weeks old were partially immobilized by ether inhalation for 10 s, and 10^5 B16-F10 cultured cells in 0.05 ml of EBSS injected 5 mm proximal to the point of entry of a 27-gauge needle. Injections were into the lateral tail vein 2 cm from the base of the tail; into the middle of the hind foot 2–3 mm beneath the skin on the lower surface after insertion in the footpad; beneath the epidermis of the medial dorsal surface of the ear 5 mm from the ear base; beneath the skin on the ventral surface of the thigh midway between knee and hip; and beneath the skin midway between the front and hind legs 1 cm to the right of the abdomen centre. After 1 h, the injected areas were removed by amputation at least 5 mm proximal to the limit of the inoculum or by excision (flank), including a 5 mm margin beyond the inoculum boundary. Surgery was performed after anaesthetizing mice with chloral hydrate; large blood vessels were ligated and heat-cauterized, and open wounds were resealed with 9-mm stainless-steel wound clips. All mice were killed 21 days later and pigmented lung colonies counted by examining excised lungs with a dissecting microscope; complete autopsies revealed grossly visible tumour growth only in the lungs. These results were obtained in duplicate experiments carried out using 10 mice per group. Values in parentheses are the range of lung colonies in positive mice.

Table 2 Effect of surgical excision on metastasis of B16-F10 s.c. transplants

Procedure	No. of mice with lung nodules	Median no. of lung nodules
2.5×10^4 – 2.5×10^5 cells s.c. (autopsied at death 4–5 weeks later)	6/95*	8 (1–18)
2.5×10^5 cells s.c. ('careful' complete excision after 14 days; killed 6 weeks later)	1/20	4
2.5×10^5 cells s.c. ('careful' incomplete excision after 14 days; autopsied at death 4–6 weeks later)	11/18	66 (19–160)
2.5×10^5 cells s.c. ('careless' complete excision after 14 days; autopsied or killed 4–6 weeks later)	12/17	53 (5–165)

B16-F10 cultured cells in 0.2 ml of EBSS were injected s.c. in the right abdominal flank as described in Table 1 legend but mice were not anaesthetized before injection. Except as otherwise noted for the first group, mice were females 8–12-weeks old. 'Careful' complete excision was carried out as described in Table 1 legend and involved removal of all visible tumour plus a border of normal tissue at least 5 mm wide without disturbing the tumour; no local tumour growth occurred in any of these mice after excision. Incomplete excision was done in the same manner but only grossly visible tumour was removed; traces of tumour remained to grow and eventually kill all these mice. 'Careless' excision was done without ligating and cauterizing blood vessels, and tumours were handled without caution during removal; local tumour growth occurred in 53% of these mice. The mean size of excised tumours was 1.04 ± 0.45 cm³, and all were richly vascularized. Values in parentheses are the range of lung nodules in positive mice.

* Included in this group were mice injected with 2.5×10^4 (0/15) and 2.5×10^5 (2/48) cultured cells, and 10^5 tumour cells purified from an i.p. transplant (1/10); male mice 8–12-weeks old injected with 2.5×10^5 cultured cells (1/10), and female mice 12–18-months old injected with 2.5×10^5 cultured cells (2/12).

(EBSS). Cell suspensions in EBSS were injected into disease-free C57BL/6 mice (Jackson Laboratory or our own colonies), matched in sex, age and weight for each experiment, within 10 min after collection⁹. Tumour cells from single-generation s.c. or i.p. transplants were purified to >90% viability and homogeneity by centrifugation on continuous-density gradients of buffered iso-osmotic metrizamide after physical separation of cells from solid tissue¹⁰. Tumour cells were prepared for injection in the same manner as cultured cells.

The B16-F10 cell line was highly selected by Fidler³ to produce large numbers of lung colonies after i.v. injection, and s.c., intradermal (i.d.) and intramuscular (i.m.) transplants of these cells are reported to metastasize spontaneously to the lungs in 20–90% of syngeneic mice, usually after excision of the growing transplant^{11–14}. With regard to metastatic activity, several of those transplantation sites are richly vascularized, and the possibility of introducing potential lung-colonizing cells directly into ruptured blood vessels during injection must be considered. When B16-F10 cells were introduced into various s.c., i.d. and i.m. sites and tumour prevented from developing at the injection site by amputation or excision of the affected areas, lung colonies developed in some mice (Table 1); in mice with growing transplants such colonies could be mistaken for spontaneous metastases. Only s.c. injection in the abdominal flank, in an area which was predetermined as relatively free of blood vessels visible at low magnification with a dissecting microscope, failed to result in some lung colonization, and this site was chosen for subsequent experiments. Similarly, surgical excision of B16-F10 transplants, while prolonging host survival and growth of pre-established micrometastases, could also allow tumour cells inadvertently to enter severed blood vessels and eventually colonize the lungs—this possibility was also examined. Tumours were excised with great care after 2 weeks of s.c. growth and lung nodules formed in <10% of mice, comparable with the incidence of nodules in mice bearing unexcised tumours (Table 2); these nodules may represent true spontaneous metastases. In contrast, careless or incomplete tumour excision resulted in extensive lung nodule formation, probably due to colonization rather than spontaneous metastasis.

With these considerations, lung colonizing and spontaneous metastatic capacities of a number of cell lines derived from the B16 melanoma were assessed. Each cell line was tested as cultured cells and as tumour cells, because the behaviour of cells growing in such diverse conditions might be different. The 'parent' B16 tumour, from which B16-LC (lung-colonizing) and B16-LM (lung-metastasizing) lines were derived, produced both lung colonies and metastases when tested as tumour cells, but cultured cells were nonmetastatic and only slightly active in colonization (Table 3). Ten B16-LC lines, of which B16-LC1 and B16-LC4 represent extremes in lung-colonizing capacities, and three lines selected by Fidler³ and Hart¹² (B16-F1, B16-F10 and B16-BL6) were not significantly metastatic (0–20%) either as cultured or tumour cells. Clones isolated from lung-colonizing lines behaved similarly. In contrast, seven B16-LM lines were metastatic in 35–100% of mice. Lines derived from tumour transplants (B16-LM1, -LM2 and -LM3) were both colonizing and metastatic, although cultured B16-LM3 cells showed little activity in either assay (Table 3). Surprisingly, lines obtained from lung metastases (B16-LM4 and B16-LM6) were potentially metastatic but at most only poorly colonizing. Twenty-five highly metastatic clones isolated from both types of metastatic line have also been tested—all were poor colonizers. A representative clone (C2) from the B16-LM4 line failed to colonize the lungs when 5×10^3 cultured cells were injected i.v. into five mice, produced few colonies (median 0, range 0–3, $n = 5$) when 5×10^4 cells were injected, but formed numerous colonies (median 47, range 19–62, $n = 5$) when 5×10^5 cells were injected. Paradoxically, it seems that cell populations that can spontaneously migrate to and grow in the lungs are less successful than many colonizing populations at proliferating in the lungs after direct i.v. introduction.

Table 3 Lung-colonizing and lung-metastasizing cell lines derived from the B16 melanoma

Cell line	Derivation	Growth form tested	No. of mice with lung colonies; median no. (range)	No. of mice with lung metastases; median no. (range)
B16	'Parent' tumour	C	10/10; 2(1-8)	0/10; 0
		T	10/10; 27(10-63)	4/10; 0(0-8)
Lung-colonizing lines				
B16-LC1	s.c. transplant (10)*	C	19/23; 1(0-7)	0/20; 0
		T	10/10; 30(12-66)	1/10; 0(0-3)
B16-LC4	Multiple lung colonies	C	10/10; 250(145->300)	0/15; 0
		T	10/10; >300(74->300)	1/10; 0(0-1)
B16-F1	(refs 1-3)	C	17/20; 11(0-25)	1/20; 0(0-1)
		T	10/10; 47(12-76)	0/10; 0
B16-F10	(refs 1-3)	C	25/25; 63(26-116)	1/25; 0(0-3)
		T	10/10; 180(91->300)	1/10; 0(0-3)
B16-BL6	(refs 12, 14)	C	10/10; 37(16-68)	2/10; 0(0-4)
		T	9/9; 110(63-165)	2/10; 0(0-5)
Lung-metastasizing lines				
B16-LM1	s.c. transplant (18)	C	14/14; 16(4-61)	8/19; 0(0-4)
		T	20/20; 32(4-120)	16/18; 4(0-14)
B16-LM2	i.p. transplant (6)	C	20/20; 20(3-68)	9/24; 0(0-12)
		T	10/10; 75(35-146)	9/10; 6(0-19)
B16-LM3	s.c. transplant (23)	C	4/15; 0(0-6)	2/15; 0(0-1)
		T	14/20; 11(0-82)	14/20; 1(0-16)
B16-LM4	Single lung metastasis	C	5/30; 0(0-7)	26/29; 5(0-17)
		T	10/10; 3(1-15)	9/10; 4(0-26)
B16-LM6	Multiple lung metastases	C	9/20; 0(0-8)	16/19; 4(0-26)
		T	9/10; 4(0-13)	9/9; 4(1-35)

B16-LC and B16-LM lines were derived from transplants, lung colonies and lung metastases originating from the 'parent' B16 melanoma (tumour tissue that, except for brief periods of serial transplantation in syngeneic mice, had been stored frozen since 1962); these lines were tested within two passages after primary isolates were frozen. Other lines (provided by Dr Isaiah J. Fidler of the Frederick Cancer Research Center and by the Mason Research Institute) were also tested within two passages. Female mice 8-12-weeks old were used in all colonization and metastasis assays, which were carried out simultaneously for each cell line. Colonization was assessed by injecting 5×10^4 cells in 0.2 ml of EBSS into the tail vein, then counting lung colonies after 21 days; metastatic activity was determined by injecting 0.25 ml of EBSS containing 2.5×10^5 cultured cells, or 10^5 tumour cells, s.c. in the abdominal flank, then counting lung nodules after 4 weeks of undisturbed tumour growth. Local tumour growth occurred in all mice receiving s.c. injections. C, cultured cells; T, tumour cells.

* Transplant generations are indicated in parentheses.

These results indicate that, based on predominant behavioural characteristics, two distinct cell populations exist in the B16 melanoma: lung-colonizing and lung-metastasizing cells. There is no evidence from analysis of clones to indicate the existence of metastatic cells with potent colonizing abilities. However, the presence of a third population, consisting of noncolonizing nonmetastatic 'null' cells, is indicated by the relatively low activity of cultured B16, B16-LC1 and B16-LM3 cells in colonization and metastasis assays. The considerable behavioural differences between lines derived from the same tumour suggest that the relative proportion of colonizing, metastatic and 'null' populations in the B16 melanoma is highly variable and dependent on growth conditions. This tumour is thus even more heterogeneous and complex than previously thought¹⁵, and the availability of cell lines and clones with such dramatic biological differences from a single tumour should facilitate efforts to elucidate the basis for metastasis.

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Hypoblastic cells can form a disk inducing an embryonic axis in chick epiblast

E. Mitrani & H. Eyal-Giladi

Embryology Section, Department of Zoology,
The Hebrew University of Jerusalem, Jerusalem, Israel

The primitive streak of the chick embryo develops from one of the two layers of cells of the stage XIII blastoderm¹, the epiblast. The other layer of cells, the hypoblast, seems to be necessary for the induction of the primitive streak and also determines its orientation²—rotation of the hypoblast by 90° is followed by a similar rotation of the embryonic axis³. After stage XIII, the hypoblast is replaced by the invaginating endoderm and plays no further part in the development of the embryo⁴. By means of a technique for disaggregation and reconstituting cells of stage XIII hypoblasts, we have been able to show that the two functions, induction and orientation are independent and that, with reconstituted hypoblasts, the orientation of the primitive streak is determined by the epiblast.

It was not clear whether the hypoblast's function is just one of dictating the future polarity of the embryo or whether it also performs a specific inductive function. In normal development the hypoblast starts to appear at stage XI. It is formed by cells from two different sources: cells from the posterior part of the marginal zone⁵ moving anteriorly, and polyinvaginating cells coming down from the central area of the one-layer blastoderm¹. At stage XIII, if the fully formed hypoblast is removed,

the remaining blastoderm can regenerate a new hypoblast, from the two above distinct sources, which is to generate axial development in the epiblast⁶. If the peripheral regions of the marginal zone and the area opaca are removed at stage XIII, the remaining part will include only the epiblast proper and the hypoblast. These two components are sufficient for development of normal embryos. If from such an operated blastoderm the hypoblast is also removed, the remaining epiblast proper will regenerate a second layer. However, although this layer will be formed in about the same time as when the marginal zone is present during regeneration, it will only be formed from poly-investigating epiblastic cells and will not be capable of producing axial development in the epiblast. Only unorganized mesoderm such as blood islands will be formed⁶. It seemed possible that in the latter case, the information concerning direction of growth, which is normally transmitted by a normal hypoblast to the epiblast, is lacking, and therefore the epiblast fails to organize the resulting mesoderm in a polarized manner.

We have conceived a model system which allows us to separate induction from polarity within the hypoblast so that the two processes can be studied independently. For this purpose we use a culture system in which individual hypoblastic cells are made to grow and to aggregate into a disk which closely resembles the original hypoblast. This reaggregated hypoblast is then layered on to the ventral surface of a normal stage XIII epiblast and the system is incubated further on a chick vitelline membrane.

In some cases care was taken to mark, on the epiblast, the posterior side of the original blastoderm with carbon particles. The conditions used to obtain appropriate hypoblast aggregates varied. A compromise had to be reached between the minimum time necessary to obtain a proper aggregate and the time at which the functional capacity of the cells is maximal. The best hypoblast aggregates were obtained after culturing the hypoblastic cells for 11 h (Fig. 1a). If the hypoblastic cells were cultured for only 5 h the aggregates formed were more difficult to remove as an intact sheet but were as functional as those obtained after 11 h. The results of the experiments in which hypoblast aggregates were layered on to the ventral surface of stage XIII epiblasts are summarized in Table 1. When reconstituted hypoblasts reaggregated for 11 h and layered on top of stage XIII blastoderms deprived of their original hypoblast were incubated further, they were able to induce development of normal embryos. Subsequent experiments were performed by layering the reconstituted hypoblasts on top of stage XIII blastoderms previously deprived of the hypoblast, the marginal zone and the area opaca (Fig. 1b, c). This ensured that the embryos obtained were not a result of regeneration of a new hypoblast from the marginal zone⁶.

In 10 of the experiments performed, the expected posterior side of the embryo was clearly marked in the epiblast. Of these 10, all developed an axis according to the previously marked direction. The degree of development in all the experiments recorded in Table 1 varied from a definite primitive streak to embryos with several pairs of somites.

Table 1 Development and orientation of embryos from epiblasts incubated with reaggregated hypoblasts

	No. of experiments	No. of blastoderms with embryonic axis	Deviation from original blastoderm axis
Epiblasts with marginal zone and area opaca	5	5 (2)	0° (2)
Epiblasts proper, no marginal zone and no area opaca	17	14 (8)	0° (8)

Numbers in parentheses indicate the number of cases in which the direction of the axis was recorded.

Clearly the position of the cells with respect to each other in the primary hypoblast is not critical in bringing about an embryonic axis in the epiblast. The fact that the embryos developed in accordance with the expected axis of the original blastoderm indicates the existence within the epiblast of sufficient information to determine the future direction of

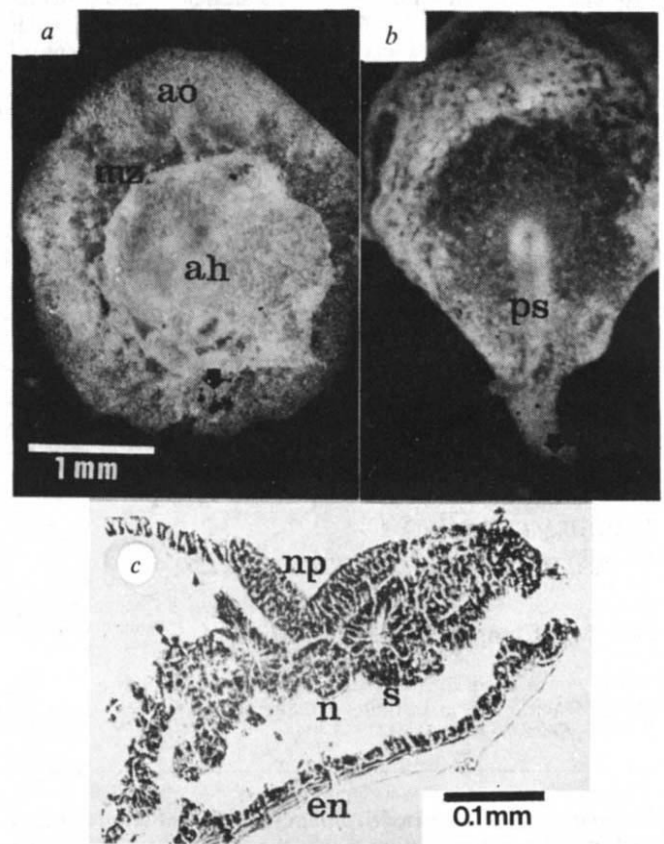


Fig. 1 a, The normal hypoblast has been replaced by the aggregated hypoblast (ah) in this stage XIII blastoderm. The marginal zone (mz) and the area opaca (ao) have been left to illustrate the relative area occupied by the aggregate. To prepare aggregated hypoblasts, normal hypoblasts were surgically removed from stage XIII blastoderms. To obtain individual hypoblastic cells, several hypoblasts were incubated in a solution containing trypsin and EDTA, transferred to the incubating medium, dissociated by gentle pipetting so that essentially a single-cell suspension was obtained, centrifuged down and resuspended in the incubating medium at a concentration of about 3×10^5 cells ml^{-1} . The incubating medium consisted of Dulbecco's modified Eagle's minimal essential medium containing 10% heat-inactivated horse serum. The cells were then incubated inside a hollow plastic cylinder whose internal diameter (3 mm) was slightly larger than that of an intact hypoblast. The cylinder of between 1 and 2 cm in height was made to stand on the lower side of a chick egg's vitelline membrane which had previously been stretched on a glass ring and suspended on chick egg albumin. The system was incubated at 37.5 °C in an atmosphere of 95% air/5% CO_2 for between 3 and 11 h. During this period the dissociated hypoblast cells within the cylinder reaggregate at the surface of the membrane and may form a one-layer disk which closely resembles the original hypoblast. The plastic cylinder was then removed, and the reconstituted hypoblast detached from the membrane with fine glass needles and layered on to the ventral side of a normal stage XIII epiblast. b, Primitive streak (ps) that developed by layering a reaggregated hypoblast on top of an epiblast proper, previously deprived of the marginal zone and the area opaca. The expected posterior side of the embryo was marked with carbon particles (arrow) on the epiblast before removal of the area opaca. c, Transverse section of an embryo that developed from an aggregated hypoblast layered on an epiblast proper. The area opaca and the marginal zone were removed before incubation. np, Neural plate; ch, notochord; s, somite; en, endoderm.

growth of the embryo. The necessity of the interaction with hypoblastic cells but not with the intact polar hypoblast indicates that a normal hypoblast is also acting as an inductor, and consequently that this induction process is indeed a specific function performed by the hypoblast.

The nature of the 'induction' process performed by a normal hypoblast is not yet known. It is unlikely, however, that the hypoblast's function is just one of a physical layer that provides some sort of cover to the denuded epiblast and/or provides non-specific anchorage or guidance for the movement of mesodermal cells. As mentioned above, even a closely associated layer, unless it contains the marginal zone-derived cells, is not sufficient to generate axial structures on a stage XIII chick epiblast.

We have therefore demonstrated that two processes which are independent of each other are necessary for the development of an embryonic axis: a determination of the direction of growth, and a specific induction process which is performed by the hypoblast on the epiblast. Although these two processes are normally both performed by the hypoblast, the second is specific to the hypoblast and can be fully operative even if information concerning the direction of growth is lacking within the hypoblast. We have also demonstrated that in fact there exists a latent

information on direction of growth stored in the epiblast which can be expressed by exposing the epiblast to a non-polarized process of induction. The direction of growth information contained in the epiblast is not dominant and is generally overridden by the stronger polarizing effect of a normal hypoblast.

It has also been shown here that a stage XIII epiblast proper can provide sufficient information to organize its own development in a polarized manner. Yet the mesoderm that develops when an epiblast proper is incubated further in the absence of hypoblastic cells, derived from a normal hypoblast, fails to show an axial pattern. It thus seems that only a certain type of mesoderm can be organized in an axial manner and that as a result of the interaction of the epiblast with the hypoblastic cells this particular type of mesoderm emerges.

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Generation of cytotoxic T-lymphocyte precursor cells in T-cell colonies grown *in vitro*

Lai-Ming Ching & Richard G. Miller

Department of Medical Biophysics, University of Toronto, and the Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Ontario, Canada M4X 1K9

The role of the thymus in T-lymphocyte differentiation remains unclear. The demonstration^{1,2} that the thymus can restrict the T-lymphocyte specificity repertoire suggests that T cells acquire specificity within the thymus. However, the demonstrations of immunocompetent helper T cells³ and cytotoxic T-lymphocyte precursor cells (CLPs)⁴ in athymic nude mice suggest that the acquisition of some T-cell reactivity may occur without the thymus. We have been using T-cell colonies grown *in vitro* as a model system for studying various aspects of T-cell differentiation in both mouse⁵⁻⁸ and man⁹. In one study⁶ we showed that CLPs can be found in T-cell colonies grown from spleen cells of normal mice, each colony containing CLPs of several different specificities. The colonies containing CLPs are not clonal, appearing to have a colony-forming unit (CFU-T) of two (perhaps three) cells⁶. Here we provide direct evidence that the CLPs are spontaneously produced in the colonies. In addition, the cells of the CFU-T were characterized with antisera directed against the cell-surface marker Thy-1, which is present on all murine T cells, and the cell-surface markers Lyt-1 and Lyt-2, which are differentially distributed on different T-cell subclasses^{10,11}. We found that the CFU-T contains both a Thy-1⁺ and a Thy-1⁻ cell, neither of which seems to carry either Lyt-1 or Lyt-2 surface markers.

T-cell colonies were grown from mouse spleen cells as described in Table 1, picked individually after 5-6 days of culture when they had reached a size of 3,000-5,000 cells, and transferred individually into microcultures where they were tested for their ability to generate cytotoxic T lymphocytes (CL) from CLPs. In the experiment shown in Fig. 1, colonies were grown from aliquots of a spleen-cell suspension which either had or had not been depleted of Lyt-2⁺ cells. As both CL and CLPs are known¹⁰ to carry the surface marker Lyt-2, depletion of Lyt-2⁺ cells ensured that any CLPs detected in the colonies actually developed there. Both sets of colonies contained CLPs

in that, after activation with the mitogen concanavalin A (Con A), they developed cytotoxic activity which could be removed by depletion of Thy-1⁺ cells. The Thy-1 marker is found on all CL but is absent or only weakly present on a fraction of other types of cytotoxic effector cells¹². Figure 1b shows that when aliquots of the spleen cells used to grow the colonies were tested in a mixed lymphocyte reaction, those depleted of Lyt-2⁺ cells failed to develop cytotoxic activity, verifying that the depletion treatment did in fact remove CLPs.

Table 1 summarizes experiments designed to test the cellular composition of the CFU-T required for CLP generation. Spleen cells were treated as indicated and then tested for their ability to produce T-cell colonies. If T-cell colonies were obtained, they were individually tested for CLP content as in Fig. 1. Spleen cells, whether depleted of Lyt-1 cells, Lyt-2 cells, both Lyt-1 and Lyt-2 cells, or nylon wool-adherent cells, grew comparable numbers of T-cell colonies of which a comparable fraction contained CLPs. The number of CLPs per colony did not vary appreciably from group to group. Removal of all Thy-1⁺ cells, however, had a different effect. Although comparable numbers of colonies were obtained, far fewer of them contained CLPs. These colonies were still T-cell colonies in that the cells were killed with anti Thy-1 plus complement (C'). The Thy-1⁺ cell had to be viable and able to proliferate as the addition of either irradiated or mitomycin C-treated spleen cells to spleen cells depleted of Thy-1⁺ cells did not increase the CLP content of the colonies. We have shown previously⁶ using stimulator cells for activation of specific CLPs in normal spleen colonies rather than Con A activation of all CLPs, that each colony contains CLPs of 10 or more different specificities. These results have been confirmed¹³ in experiments in which CLPs were activated using Con A but only CL specific for a particular target were measured.

The above data are consistent with the following model. The central component of the CFU-T is a Thy-1⁻, Lyt-1⁻, Lyt-2⁻ cell which can give rise to Thy-1⁺ cells in a colony. If the CFU-T also includes a Thy-1⁺ but Lyt-1⁻, Lyt-2⁻ cell, CLPs are also generated in the colony. Although this latter cell seems to have an unusual marker distribution, such a distribution has been previously reported¹⁴. It is necessary to add the qualification that cells found to lack a marker in a complement-mediated cytotoxicity test may still have this marker but either at a low level or displayed in a manner such that complement-mediated lysis cannot occur¹¹.

A crucial unresolved question is the role of the thymus in generating the cells required for the CFU-T. Previous studies have shown that colonies containing Thy-1⁺ cells can be grown

Table 1 Cellular composition of CFU-T for CLP generation

Cells plated	Colonies per 5×10^5 cells plated	% Colony cells killed by anti-Thy-1 and C'	Colonies with CLPs % Total colonies
Spleen	27 $\pm$ 10	88	432/564 (77)
Nylon wool non-adherent spleen	68 $\pm$ 21	N.D.	84/96 (88)
Lyt-1 depleted spleen	22 $\pm$ 8	100	17/24 (71)
Lyt-2 depleted spleen	29 $\pm$ 11	85	67/83 (81)
Lyt-1 and Lyt-2 depleted spleen	21 $\pm$ 6	N.D.	12/12 (100)
Thy-1 depleted spleen	59 $\pm$ 26	98	6/238 (2.5)
Thy-1 depleted spleen plus mitomycin C-treated spleen	42 $\pm$ 10	N.D.	1/47 (2.1)
Thy-1 depleted spleen plus irradiated spleen	77 $\pm$ 27	N.D.	1/47 (2.1)

Colonies were grown by culturing 5×10^5 viable cells from RNC-*nu*/+ mice treated as indicated, in 1 ml of α -medium²³ supplemented with 0.8% methylcellulose, 40% PHA-LCM, 20% fetal calf serum, 5×10^{-5} M 2-mercaptoethanol and 0.3 mg ml⁻¹ L-glutamine as previously described⁶. The PHA-LCM is conditioned medium prepared by incubating human peripheral blood leukocytes (10^6 cells ml⁻¹) for 7 d in α -medium containing 1% PHA and 10% fetal calf serum¹⁶. Colonies were tested for CLP content as indicated in Fig. 1 except that in some experiments 2×10^5 irradiated (1,500 rads) RNC-*nu*/+ spleen cells were used in place of RNC-*nu* spleen cells and that the cultures were not always supplemented with Con A conditioned medium, in which case peak response was on day 4 and cytotoxic activity per positive colony only half as great. All procedures gave equivalent results with respect to the fraction of colonies containing CLPs¹³. Nylon wool-adherent cells were removed by the procedure of Julius *et al.*²⁴. Antiserum treatments on spleen cells were as in Fig. 1. The antisera used, with the addition of hybridoma anti-Lyt-1, clone W3, were also as in Fig. 1. Cells (10^7 per ml) were treated with mitomycin C (25 μ g ml⁻¹ in medium) by incubation for 20 min at 37 °C. Treatment of colony cells with anti-Thy-1 and C' was carried out by incubating 10^4 pooled colony cells with anti-Thy-1 (1:50 final dilution) and rabbit complement (1:15 final dilution) for 45 min at 37 °C. Cell viability was assessed by eosin exclusion.

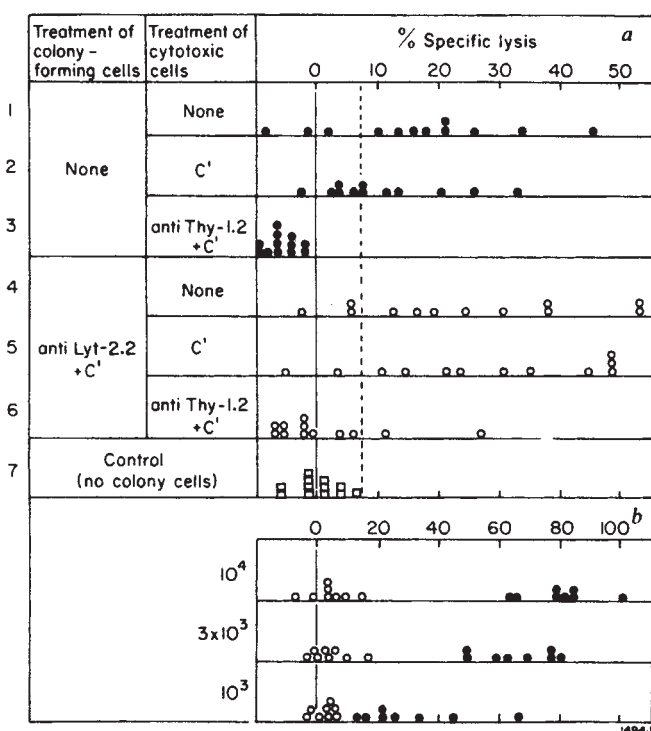


Fig. 1 a, Spleen colonies were grown from RNC *nu*/+ spleen cells which either had (○) or had not (●) been treated with anti-Lyt-2 plus complement and were then tested for their ability to produce CL from CLPs by transferring them individually into microwells along with 2×10^5 RNC. *nu* spleen cells in 0.2 ml of α -medium containing 2 μ g ml⁻¹ Con A and supplemented with 10% fetal calf serum, 5×10^{-5} M 2-mercaptoethanol and 10 mM HEPES buffer as described previously⁶. The mitogen Con A activates all CLPs regardless of specificity²⁵. On day 3, 0.1 ml from each culture was replaced with 0.1 ml of conditioned medium from mouse spleen cells stimulated for 18 h with 5 μ g ml⁻¹ Con A¹⁸ and on day 5, cultures were assayed for cytotoxic activity by adding ⁵¹Cr-labelled DBA/2 splenic Con A blasts in the presence of PHA (final dilution 1:60) as described previously⁶. The PHA seems to stick CLs to target cells so that a CL can kill any target cell irrespective of whether it can recognize it²⁵. Depletion of Lyt-2 cells was done by incubating 10^7 spleen cells with 50 μ l anti-Lyt-2 hybridoma antiserum (clone 19-178) in a total volume of 0.5 ml medium at 4 °C for 30 min, followed by a wash and 45 min incubation at 37 °C with 0.5 ml rabbit complement (1:15 dilution) (Cedarlane Laboratory). Treatment with anti-Thy-1 was done by adding anti-Thy-1 antiserum (1:50 final dilution) and rabbit complement (1:15 final dilution) to the cultures for 45 min at 37 °C. Cultures were washed four times before the addition of ⁵¹Cr-labelled target cells. Purified rabbit anti mouse Thy-1 glycoprotein antibody was used²⁶. b, Three concentrations of the spleen cells used to grow the colonies tested in a, both untreated (●) and anti-Lyt-2 plus complement treated (○), were cultured with 3×10^5 irradiated (1,500 rad) DBA/2 spleen stimulators and 10^5 RNC. *nu* spleen cells and assayed on day 5 for cytotoxicity against DBA/2 splenic Con A blasts.

from Thy-1⁺, presumably pre-thymic, progenitor cells in nude spleen⁵, bone marrow⁸ and long-term bone marrow cultures⁷. All these colonies seem to contain T-helper cell activity and those of bone marrow origin also contain Thy-1⁺ cells which are apparently involved in inactivating certain self-reactive CLPs⁸. In common with colonies grown from normal spleen cells depleted of Thy-1⁺ cells, none of these colonies contains CLPs. We hypothesize that the Thy-1⁺ component of the CFU-T from normal spleen required to generate CLPs in colonies is a post-thymic cell. It might provide an equivalent to the thymus microenvironment required for CLP generation or itself be a post-thymic CLP precursor cell. According to Stutman¹⁵, immunologically incompetent thymus-processed precursor cells are exported from the thymus to the periphery. The maturation of these post-thymic precursors into competent T cells requires specific interaction with inducer cells in the periphery. Thus, the CFU-T may represent the inducer, post-thymic precursor cell pair.

Growth of the T-cell colonies discussed here has an absolute requirement for a phytohaemagglutinin (PHA)-induced supernatant factor which we call PHA-LCM (see Table 1 legend, refs 16, 17). Although made in much the same manner as IL-2 (also called co-stimulator or T-cell growth factor (TCGF)¹⁸⁻²¹), the incubation period is much longer (7 days compared with 18-48 h). Neither IL-2 nor PHA alone will support growth of T-cell colonies of the type described here²². Thus, there seems to be factor(s) in the PHA-LCM which allows the *in vitro* growth and differentiation of more immature T cells than those T cells which can respond in the mixed lymphocyte reaction or can be made to grow in the presence of IL-2. Although the development of functional T cells in the colonies is certainly extra-thymic, this development should probably not be considered as thymus independent, even for those types of colonies for which the CFU-T contains no Thy-1⁺ cells: the production of PHA-LCM is very likely to depend on the presence of T cells. We conclude that the T-cell colonies described here provide a valuable *in vitro* model system for investigating questions concerning T-cell differentiation and development of the CLP specificity repertoire not approachable with other available *in vitro* techniques.

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Induction of light chain expression in a pre-B cell line by fusion to myeloma cells

Sylvia C. Riley, Emily J. Brock & W. Michael Kuehl

Department of Microbiology, University of Virginia, School of Medicine, Charlottesville, Virginia 22908, USA

Pre-B cells, the first cells in the B-lymphocyte differentiation pathway which express immunoglobulin¹, have recently been shown to express cytoplasmic μ heavy chain (H) but not light chain (L)^{2,3}. If, as is believed, pre-B cells are the precursors of immature B lymphocytes, which express surface IgM⁴⁻⁷, the differentiation of pre-B cells to immature B lymphocytes must be accompanied by the expression of light chains. In this case, it should be possible for the progeny of a single pre-B cell to express a variety of light chains in association with the same heavy chain. We have tested this hypothesis by hybridizing a pre-B cell line 18-81 expressing only cytoplasmic μ chains⁸ with variant myeloma cells which do not express light chains. Hybridization of B-lymphoma cells with myeloma cells usually produces a hybrid with the phenotype of the more differentiated parent⁹⁻¹¹. In this case, the fusion resulted in the induction of light chain expression from the 18-81 genes and we have been able to demonstrate that independent hybrids express different light chains, in accordance with the hypothesis that a pre-B cell committed to expression of a single μ heavy chain can generate progeny expressing different light chains¹².

We used a cloned, virus-transformed pre-B-cell line (18-81) which has been shown to express cytoplasmic μ heavy chain (μ_c) but no light chain. Our subline of 18-81 synthesizes up to 1% of its cell protein as μ_c in the absence of detectable light chain synthesis. A hypoxanthine guanine phosphoribosyl transferase (HGPRT)-negative clone of 18-81, 18-81.T5 (μ_c^+ , L⁻), was isolated and hybridized to CBOHC (γ_2^+ , L⁻), a thymidine kinase(TK)-negative variant of myeloma CBO(γ_2^+ , κ^+) which, like 18.81.T5, does not express light chain. Immunoglobulin expression was analysed by SDS-polyacrylamide gel electrophoresis of immunoprecipitates isolated from ³⁵S-Met-radiolabelled postnuclear supernatants. Immunoglobulin is expressed by 75% of presumptive hybrids; the reason for lack of immunoglobulin expression by hybrids is unclear but could be due to chromosome segregation. All immunoglobulin-expressing hybrids examined synthesize either or both of the μ and γ_2 heavy chains of the parent cells and 13 (40%) of these express a κ light chain which is not produced by either of the parent cells (Fig. 1). Expression of λ light chain is not detected in these

hybrids¹³. We tested for λ expression using rabbit antisera specific for mouse λ I light chain. The hybrids were not tested for λ II or λ III subclasses. To show that expression of κ light chain by these hybrids is not due to re-expression of the wild-type CBO κ light-chain gene, the CBO and hybrid light chains were analysed on an isoelectric focusing gel (Fig. 2). Light chains from three different CBOHC $\times$ 18-81.T5 hybrids (Fig. 2, lanes c-e) focused at a different pH range than the light chain from CBO (Fig. 2f). In addition, Southern blots of CBO and CBOHC DNA indicate that the expressed CBO κ light chain gene is deleted from the CBOHC variant (M. Kuehl, unpublished result). Thus expression of κ light chain by these hybrids is not due to re-expression of the wild-type CBO myeloma κ light-chain gene. It must therefore be due to expression of another myeloma κ light-chain gene or a pre-B cell κ light-chain gene. To determine whether the κ light chain expressed in pre-B cell $\times$ myeloma hybrids is coded for by the pre-B cell genome, a TK-negative clone of 18-81, 18-81.B4 (μ_c^+ , L⁻), was isolated and hybridized to NT₂(H⁻, L⁻, F_{CL}⁺), an HGPRT-negative mutant of myeloma variant NP₂ which does not express heavy or light chains¹⁴. NT₂ contains only the aberrantly rearranged κ constant region (C _{κ}) gene which is expressed as a 12,000-molecular weight (MW) κ

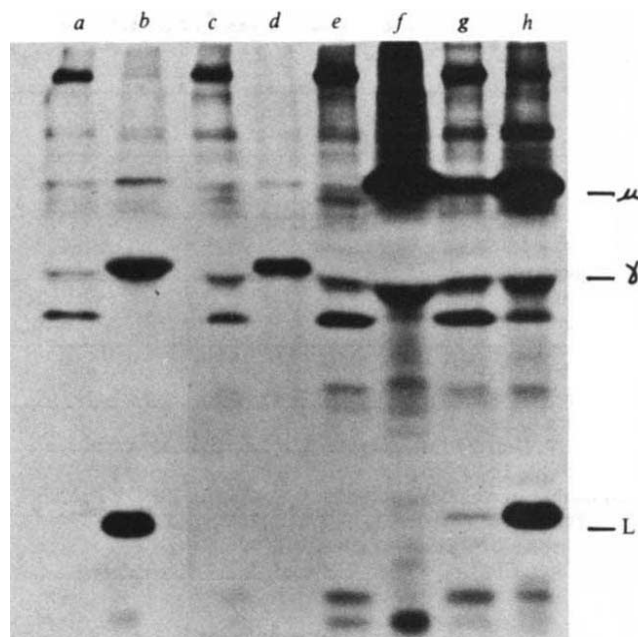


Fig. 1 SDS-polyacrylamide gel electrophoretic analysis of immunoglobulin synthesized by CBOHC myeloma, 18-81.T5 pre-B cell and CBOHC $\times$ 18-81.T5 hybrids. The 18-81.T5 HGPRT-negative variant of 18-81 was selected in $6 \mu\text{g ml}^{-1}$ 2-amino-6-mercaptopurine (6-TG). The CBO (γ_2^+ , κ^+) TK-negative variant of C1.3 was selected in $30 \mu\text{g ml}^{-1}$ 5-bromo-2'-deoxyuridine (BUdR) after mutagenesis with $1 \mu\text{g ml}^{-1}$ N-methyl-N'-nitro-N-nitrosoguanidine. The CBOHC (γ_2^+ , L⁻) variant clone was isolated from CBO. 18-81.T5 and CBOHC were fused using 35% polyethylene glycol (PEG) by methods described by Gefter *et al.*²⁰ and modified by Clafin *et al.*²¹. The parent cells were washed free of serum and mixed in equal numbers (10^7 of each) in 35% PEG. The cells were pelleted twice by low-speed centrifugation, resuspended in growth medium plus HAT (hypoxanthine, aminopterin and thymidine), and cloned into 96-well microtitre plates. The cells and hybrid clones were metabolically labelled for 2 h with ³⁵S-Met, washed and lysed in 1% NP-40 (ref. 22). Postnuclear supernatants were indirectly immunoprecipitated with rabbit antibodies to mouse μ , γ_2 , κ and λ chains and goat antiserum to rabbit immunoglobulin²³. After dissolving the precipitate in SDS the immunoprecipitates were reduced, electrophoresed on 11.25% SDS-Tris-glycine-polyacrylamide gels²⁴, and analysed by photofluorography²⁵. a, c, e, g, Normal rabbit serum; b, d, f, h, rabbit anti-MPC11 (γ_2 , κ) + anti-MOPC104 (μ , λ); a, b, hybrid 1; c, d, CBOHC; e, f, 18-81.T5; g, h, hybrid 2.

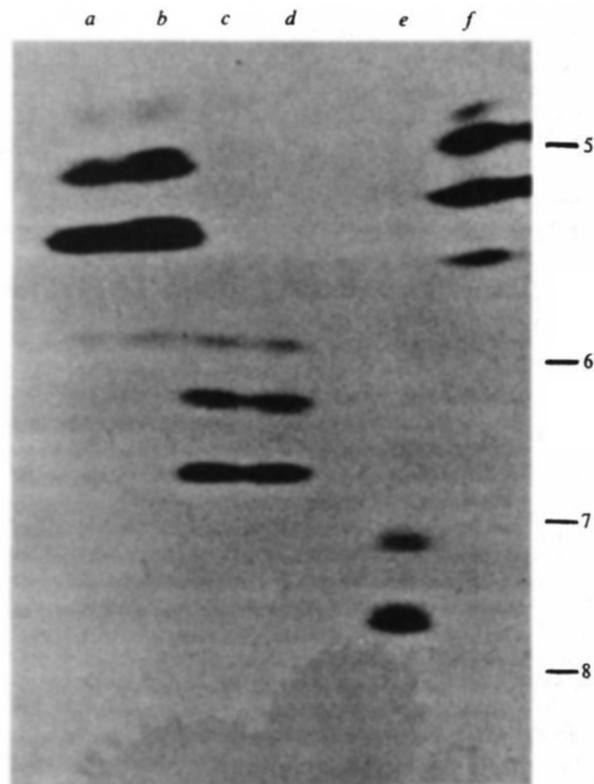


Fig. 2 Isoelectric focusing analysis of light chain from CBO cells and NT₂ × 18-81.B4 and CBOHC × 18-81.T5 hybrids. Immunoprecipitates using rabbit antisera specific for mouse κ light chain and goat antiserum to rabbit immunoglobulin were prepared from ³⁵S-Met-labelled postnuclear supernatants, reduced and electrophoresed on 9% SDS-polyacrylamide slab gels as described in Fig. 1 legend. A small strip which contained light chain was cut from the gel, equilibrated in 7M urea, 1% NP-40 and 2% ampholines, then focused on an isoelectric focusing gel (4.7% acrylamide, 0.12% *N,N*-methylene bisacrylamide, 16.6% glycerol, 7M urea, 1% NP-40, 2.5% carrier ampholines (pH 3.5–10), and 0.033% ammonium persulphate) according to the procedure of Burrows *et al.*². The gel was analysed by photofluorography. *a*, NT₂ × 18-81.B4 hybrid 1; *b*, NT₂ × 18-81.B4 hybrid 2; *c*, CBOHC × 18-81.T5 hybrid 3; *d*, CBOHC × 18-81.T5 hybrid 4; *e*, CBOHC × 18-81.T5 hybrid 2; *f*, CBO.

light chain constant region fragment (F_{CL}) in all MPC11 myeloma cells and MPC11 variants^{15,16}. Five out of six presumptive hybrids synthesize the μ and F_{CL} chains of the parent cells, but two (40%) of the immunoglobulin-expressing hybrids synthesize a full-sized κ light chain, which migrates slightly more rapidly on an SDS gel than the MPC11 κ light chain (Fig. 3). Expression of λ light chain is not detected in these hybrids¹³. Because the hybrids which express a *de novo* full-sized κ chain also express F_{CL} , and the only C_{κ} gene present in the NT₂ myeloma parent is expressed as F_{CL} ^{15,16}, the synthesis of a full-sized light chain cannot be due to expression of a myeloma κ light chain gene. Therefore, the κ light chains expressed by the pre-B cell × myeloma hybrids are coded for by the pre-B cell genome.

If the 18-81 pre-B cell is already committed to expression of a particular light chain, then the same light chain should be expressed in all myeloma × pre-B cell hybrids. However, when the κ light chains from five different pre-B cell × myeloma hybrids were compared by isoelectric focusing (Fig. 2) and by peptide mapping of leucine-radiolabelled tryptic-chymotryptic peptides (data not shown), at least three different κ light chains were found. Of the five hybrids examined for the isoelectric

pattern of the newly expressed light chain (Fig. 2), there were two pairs of hybrids (NT₂ × 18-81.B4, Fig. 2*a, b*, and CBOHC × 18-81.T5, Fig. 2*c, d*) with indistinguishable light chains. However, the conclusion that the same light chain is expressed in independent hybrids is not justified because in each case the pair of hybrids expressing apparently identical light chains was from the same hybridization experiment.

Because somatic cell hybrids between myeloma cells and 18-81 express at least three different light chains, induction of light chain expression is probably not due to enhanced expression of a rearranged light-chain gene in the original 18-81 clone. This conclusion is supported by Southern blots, using *Bam*HI-*Xba*I fragment derived from an embryonic C_{κ} genomic clone as the probe¹⁷. The C_{κ} genes in 18-81, 18-81.B4 and 16 subclones of 18-81.B4 are on a 13-kilobase fragment which co-migrates with the 13-kilobase embryonic *Bam*HI fragment present in splenic DNA (data not shown). As DNA rearrangement to form active κ light-chain genes has apparently not occurred in the original 18-81 clone, there are two other possibilities: first, light-chain gene rearrangement could occur spontaneously in the 18-81 population during culture (that is, before somatic-cell hybridization). Second, rearrangement and expression of an 18-81 light-chain gene could also occur during or following hybridization of 18-81 with a myeloma cell. When 24 subclones of 18-81.B4 were analysed for light chain synthesis, none of the subclones expressed κ or λ light chain (D. Pinney and S. Riley, unpublished results). Therefore, if the light chain is rearranged spontaneously in 18-81 cells, fusion of 18-81 with a myeloma cell is required to induce detectable light chain synthesis. This

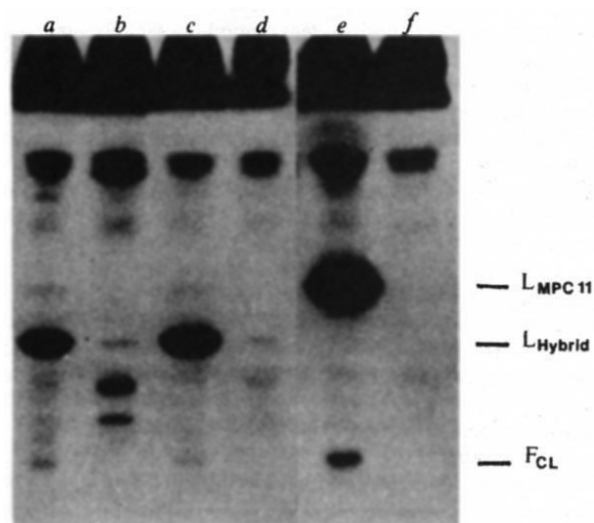


Fig. 3 SDS-polyacrylamide gel electrophoretic analysis of immunoglobulin synthesized by NT₂ myeloma × 18-81.B4 pre-B cell hybrids. The 18-81.B4 TK-negative mutant of 18-81 was selected in 30 μ g ml⁻¹ BUdR after mutagenesis with 125 μ g ml⁻¹ ethylmethanesulphonate. 4T00.A(H⁻, κ ⁺, F_{CL} ⁺) is a spontaneous subline of 4T00 which no longer synthesizes detectable heavy chain. NT₂ (H⁻, L⁻, F_{CL} ⁺) is an HGPRT-negative mutant of NP₂. 4T00 (ref. 20) and NP₂ (ref. 14) were derived from the MPC11 myeloma cell line. F_{CL} is expressed in low levels in all MPC11 cell lines and its variants but is not expressed in other myeloma or 18-81 cells (data not shown). Immunoprecipitates were prepared from ³⁵S-Met-labelled postnuclear supernatants, reduced, electrophoresed on a 15% SDS-Tris-glycine-polyacrylamide gel, and analysed by photofluorography as described in Fig. 1 legend. Minor non-immunoglobulin contaminants are seen in the specific and nonspecific immunoprecipitates because the photograph was overexposed to show F_{CL} . *a, c, e*, Rabbit anti-MPC11 (γ_2 , κ) + anti-MOPC104 (μ , λ); *b, d, f*, normal rabbit serum; *a, b*, NT₂ × 18-81.B4 hybrid 1; *c, d*, NT₂ × 18-81.B4 hybrid 2; *e, f*, 4T00.A.

possibility may be supported by the results of Rosenberg *et al.*^{18,19} who have observed that lipopolysaccharide (LPS) can induce enhanced μ heavy chain expression and *de novo* light chain expression in some of their 18–81 sublines; although they provide no evidence to show whether LPS is inducing rearrangement of light-chain genes or is only enhancing expression of spontaneously rearranged light-chain genes. In contrast to their results, LPS has no effect on μ heavy chain expression or in inducing light chain expression in our 18–81 subline or the 24 subclones described above (D. Pinney, unpublished results).

Regardless of whether light chain rearrangement occurs spontaneously or as a consequence of cell fusion, the expression of different light chains by different hybrids suggests that when the pre-B cell light chain is expressed in 18–81 myeloma hybrids, the 18–81 germline C_{κ} gene can be rearranged to at least three different κ light-chain variable region (V_{κ}) genes. Southern blots of DNA from pre-B cell myeloma hybrids provide direct evidence for this rearrangement. One hybrid from each of three different κ light-chain groups, as defined by isoelectric focusing (Fig. 2), was analysed for rearrangement of κ light chains by Southern blots, using *Bam*HI-restricted genomic fragments and a nick-translated *Bam*HI-*Xba*I fragment derived from an embryonic C_{κ} genomic clone as the probe¹⁷. Each hybrid lacks the 13-kilobase C_{κ} fragment but contains a new rearranged C_{κ} which is not found in either 18–81 pre-B cells or CBOHC, CBO, NT₂ or 4T00.A myeloma cells. The *de novo* rearranged C_{κ} fragments in each of the three hybrids has a unique size compared with the *de novo* C_{κ} fragments in the other two hybrids (data not shown).

The development of a pre-B cell from a stem cell involves the rearrangement and expression of the μ heavy-chain gene. Rearrangement of a V_L gene to a C_L gene could occur at the same time or after rearrangement of the μ heavy-chain gene. In the former, expression of the rearranged light-chain gene would occur later than expression of the μ heavy-chain gene. In the 18–81 pre-B cell line light-chain genes are not rearranged, although our results indicate that they can be rearranged and expressed. This phenotype may be a result of transformation by Abelson virus, but it probably reflects the phenotype of a pre-B cell population in murine bone marrow, because cells expressing μ heavy chain and no light chain have been found in normal bone marrow². It has been suggested that the asynchronous onset of μ and light-chain synthesis could be involved in the expression of antibody diversity². That is, a pre-B cell selects a particular V_H gene and rearranges it next to the C_{μ} gene. The progeny of this pre-B cell may then select a V_L gene to translocate it next to a C_L gene. In this way a rapidly proliferating pre-B cell which expresses a specific heavy-chain V region is the precursor of cells which express different light chains along with this heavy chain. Our results strongly support this hypothesis.

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Reaction of 5-iodonaphthyl-1-nitrene with the IgE receptor on normal and tumour mast cells

David Holowka*, Carlos Gitler†, Tuvia Bercovici† & Henry Metzger*

* Section on Chemical Immunology, Arthritis and Rheumatism Branch, NIAMDD, NIH, Bethesda, Maryland 20205, USA

† Department of Membrane Research, The Weizmann Institute of Science, Rehovot, Israel

Mast cells, basophils and a tumour analogue—rat basophilic leukaemia (RBL) cells—have a surface glycoprotein (R_e) which specifically binds monomeric immunoglobulin E (IgE)¹, and aggregation of R_e causes secretion^{1–3}. When isolated from non-ionic detergent extracts of surface-labelled RBL cells by IgE-specific immunoprecipitation, R_e appears as a 50,000 (50 K) to 60,000 (60 K) molecular weight (MW) band on electrophoresis in polyacrylamide gels in SDS (SDS-PAGE)^{4,5}. Likewise, only a 50 K component is observed when the polypeptide that binds IgE is isolated by affinity chromatography in conditions which prevent aggregation of the IgE^{6–8}, even when intrinsically labelled R_e is studied⁹. To determine how R_e is inserted into the plasma membrane, we reacted RBL cells with the photolysable hydrophobic reagent 5-iodonaphthyl-1-azide (INA), which preferentially labels the intramembranous segments of several intrinsic membrane proteins^{10,11}. We report here that, surprisingly, the label was found, not on the 50 K glycopeptide, but only on a 30 K component which other studies suggest is a subunit of R_e (refs 9, 12).

RBL cells from the 2H3 subline were grown as described previously¹³. In some experiments cells were first incubated with IgE. Before reaction with INA, the cells were washed to remove albumin present in the serum-containing incubation medium, with which the INA can react^{10,11}. Normal rat mast cells were saturated with IgE and isolated from peritoneal washings of Sprague–Dawley rats as described by Holgate *et al.*¹⁴, omitting the final gradient step.

Radioiodinated INA was prepared on the day of the experiment from 5-amino-1-azidonaphthalene¹⁰. Solutions containing INA were irradiated at 25–30 °C in a water-jacketed 1-cm Pyrex cuvette with a HB0 200W 14 Osram mercury lamp at a distance of 10 cm. The light was focused with a fused quartz lens and passed through Corning CS7-60 and 054 filters to yield a band centred at ~350 nm with a halfwidth of 60 nm. In all the experiments 10^7 cells in phosphate-buffered saline, pH 7.4, were irradiated for 8 min in five 2-ml batches in 3 μ M INA (specific activity ~1 Ci mmol⁻¹), 0.02% gelatin and 10 mM neutralized glutathione. The cells were then diluted twofold in medium containing 20% fetal calf serum and were ≥80% viable as judged by dye exclusion. The cells were washed twice and then solubilized with non-ionic detergent. IgE was added to the extracts from those cells which had not been previously reacted with it.

Figure 1 shows an experiment in which ¹²⁵I-INA was photolysed in the presence of RBL cells preloaded with IgE. The major band of ¹²⁵I counts had an apparent MW of 30 K. No discrete ¹²⁵I peaks occurred where a band from surface-labelled

cells appears (50 K), or where the ^{131}I label on receptor-bound IgE appeared in this gel—at 80 K and 24 K. Extracts from cells incubated with IgE after reaction with INA gave indistinguishable results. Control (human IgE) precipitates contained only very low molecular weight labelled material which may represent phospholipids¹¹ or nonspecific small peptides.

The 50 K glycopeptide component of R_e is associated specifically and stoichiometrically with a 30 K polypeptide after intact RBL cells or detergent extracts of the cells are cross-linked with dimethyl-3,3'-dithiobispropionimidate (DTBP)¹². We tested whether the 30 K component labelled with INA might represent the same polypeptide. Cells were incubated with ^{125}I -labelled IgE amidinated to prevent its cross-linking to the receptor by DTBP at a later step¹². The washed cells were reacted with ^{131}I -labelled INA, the detergent extract reacted with DTBP and immunoprecipitates analysed both before and after reduction with 0.25 M 2-mercaptoethanol (Fig. 2). The

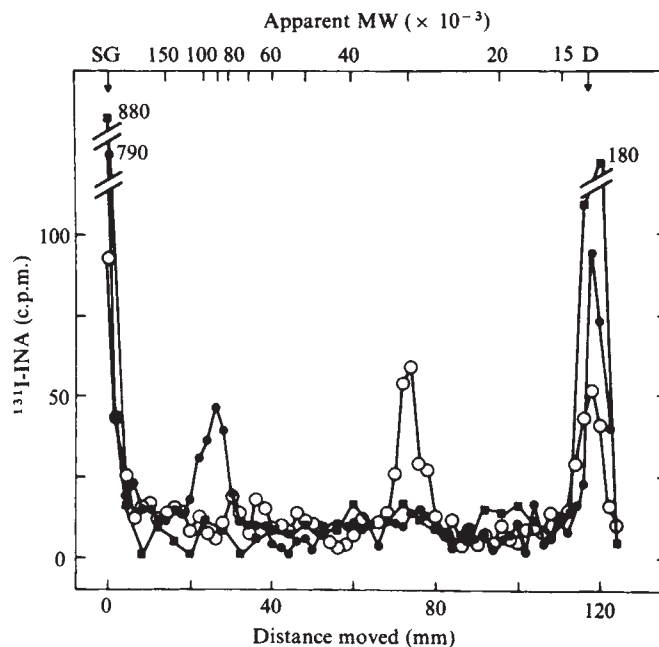


Fig. 2 SDS-PAGE patterns of immunoprecipitates from a Triton X-100 extract of RBL cells reacted with ^{131}I -INA after loading with amidinated ^{125}I -IgE. The supernatant from a 50,000g centrifugation was reacted with DTBP in two doses over 4 h, giving a final concentration of 10 mM. The cross-linking reagent was quenched with 100 mM ammonium acetate and the extract was then immunoprecipitated as described in Fig. 1 legend. ■, Control precipitate; ●, ○, anti-rat IgE precipitates. ○, Reduced sample; ●, ■, unreduced samples. The samples were electrophoresed on a 12% polyacrylamide gel as described elsewhere¹². SG, stacking gel; D, bromophenol blue dye front.

the control precipitates also, and was generally largely absent after reduction.

Labelling of R_e on normal rat mast cells gave comparable results (Fig. 3). Two peaks were observed in the specific precipitate only—a major peak at 32 K and a smaller one at 23 K. We have evidence⁹ that the latter is a proteolytic fragment of the 30 K component.

R_e behaves like an integral membrane protein: it requires non-ionic detergents to be solubilized and its partial specific volume suggests that it binds detergent (or lipids)¹⁵. Its resistance *in situ* to proteolysis¹⁶ and its failure to be surface-labelled

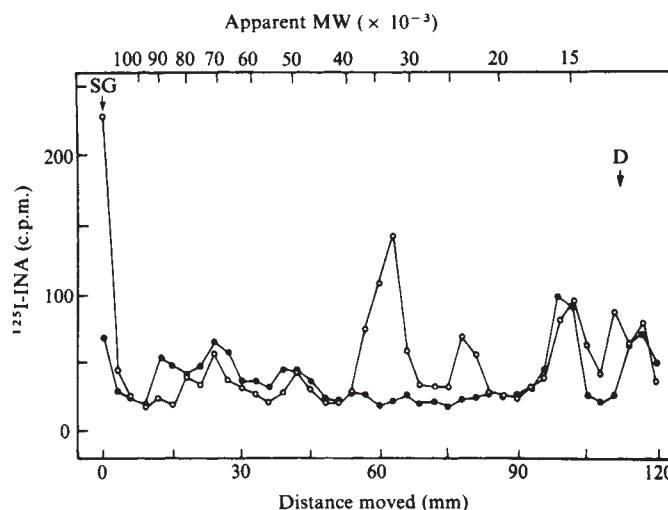


Fig. 3 SDS-PAGE patterns of INA-labelled R_e from normal rat mast cells (~90% purity) preincubated with rat IgE. A Triton X-100 extract of the cells was immunoprecipitated as in Figs 1 and 2. ●, Human IgE-anti-human IgE 'clearing' precipitate; ○, specific anti-rat IgE precipitate. The SDS eluates were reduced before electrophoresis on a 12.5% polyacrylamide gel.

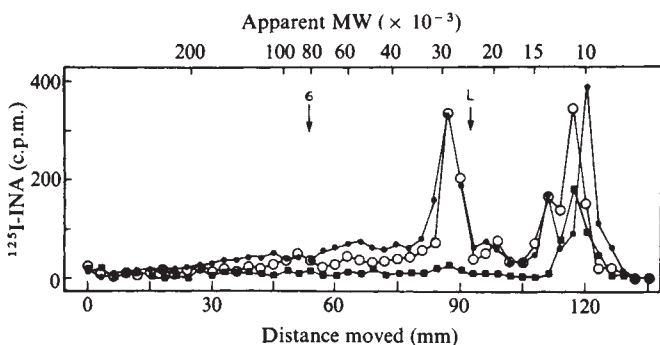


Fig. 1 SDS-PAGE patterns of immunoprecipitates from Triton X-100 extracts of cells labelled with ^{125}I -INA. Cells were lysed with 0.5% detergent in borate-buffered saline (pH 8.5) containing 0.5 mM phenylmethylsulphonyl fluoride and 0.5 units ml^{-1} aprotinin. After centrifugation at 50,000g for 30 min the extracts were diluted to 0.25% detergent and reacted with human IgE and anti-human IgE plus a *Staphylococcus aureus* Cowan preparation. The supernatant from this immunoprecipitation was then reacted with anti-rat IgE plus *S. aureus* Cowan in a similar manner. The precipitates were washed, eluted with 2% SDS and then reduced in 0.25 M 2-mercaptoethanol. ■, Extract from cells reacted with IgE before reaction with INA and precipitated with anti-human IgE and human IgE as a control. ●, Supernatant from precipitation in (■), precipitated with anti-rat IgE. ○, Extract from cells reacted with INA before addition of IgE and precipitated with anti-rat IgE (after a human IgE-anti-human IgE 'clearing' precipitation). The arrows at 80 K and 24 K are the positions at which the peaks of the ^{125}I -labelled ϵ and light chains of the rat IgE appeared. Electrophoresis was carried out on a 2.5–27% Isophore gradient gel (Isolab) as described elsewhere¹⁷.

reduced sample showed label at an apparent MW of 30 K but none where surface-labelled receptor appears on such gels (~55 K). The unreduced sample showed only a single band for those ^{131}I counts which penetrated the stacking gel, having an apparent MW of ~90 K, exactly where cross-linked R_e from surface-labelled cells appears¹². In matched non-cross-linked samples label was observed in a 30 K but not in a 50–60 K component.

As 1 mol of R_e binds 1 mol of IgE^{1,8}, we could compare the recovery of ^{131}I -INA-labelled components relative to the ^{125}I -IgE in the same samples. Equivalent amounts of the 30 K component were found for the reduced samples, both cross-linked and non-cross-linked. A lower amount was seen in the 90 K band of the cross-linked unreduced sample and is probably accounted for by incomplete penetration of the cross-linked receptor into the gel, which has occasionally been observed¹². However, most of the counts which failed to penetrate the stacking gel are not due to cross-linked receptor; such material, was seen variably, when it was observed it was always present in

when IgE is bound to it⁴ are also consistent with a substantial proportion of the receptor being within the plasma membrane or on its cytoplasmic side. The failure of the 50 K subunit to become labelled may result from its having only a short intramembranous segment; this segment may be interacting tightly with the 30 K subunit or membrane lipids, or its amino acid residues may be relatively unreactive. Other studies suggest preferential labelling of unsaturated over saturated lipids by INA¹¹ and preferential labelling of certain amino acid side chains is possible. Alternatively, the 50 K component may be anchored to the membrane solely through its interaction with the 30 K component.

Cross-linking studies had suggested that R_e might have two subunits¹². However, cross-linking reagents may fail to reveal associations because they depend on the apposition of suitably reactive residues. The present study shows that labelling with INA and analogous compounds may provide an alternative method for revealing presumptive subunits of membrane proteins which are undetectable by surface labelling and which may be lost during the rigorous purification often required when intrinsic labelling is used.

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Expression of the *E. coli uvrA* gene is inducible

Cynthia J. Kenyon & Graham C. Walker

Biology Department, Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139, USA

UvrA⁺-dependent excision repair^{1,2} is one of the most important systems in *Escherichia coli* for repairing UV-induced pyrimidine dimers¹ and a variety of other forms of DNA damage^{3,4}. The *uvrA* protein acts in conjunction with the *uvrB* and *uvrC* gene products to introduce a nick at the site of a DNA lesion and thus initiate the repair process¹. We have recently used the Mud(Ap, *lac*)⁵ operon fusion vector to identify a set of genes whose expression is induced by DNA damage. One Mud(Ap, *lac*) insertion mapped at the *uvrA* locus and made the cells sensitive to UV light. In this fusion strain, β -galactosidase expression was induced by DNA-damaging agents in a *recA*⁺*lexA*⁺-dependent fashion⁶. We were surprised by this result because *uvrA*⁺-dependent excision repair is observed both in cells in which protein synthesis has been inhibited⁷ and in *recA*⁺ and *lexA*⁺ cells⁸, findings which have led to the conclusion that the *uvrA* gene product is constitutively expressed and not under the control of the complex *recA*⁺*lexA*⁺ regulatory circuitry⁹ (see below). We have investigated this possibility further and describe here the generation and characterization of a set of fusions of the *lac* genes to the promoter of the *uvrA* gene. We confirm that the *uvrA* gene product is induced by DNA damage in a *recA*⁺*lexA*⁺-dependent fashion.

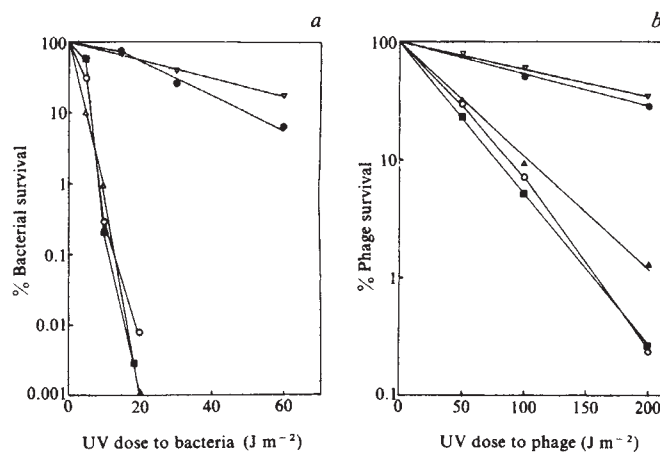


Fig. 1 *a*, Survival after UV irradiation. Fusion strains were grown in supplemented M9 minimal media¹¹ to a density of 1×10^8 cells ml⁻¹. Aliquots were UV-irradiated as indicated. Survival was determined by plating appropriate dilutions on supplemented M9 plates. *b*, Host cell reactivation. λ cI857 was irradiated in 10 mM MgSO₄. 0.1 ml diluted phage was preadsorbed to 0.1 ml of an exponential culture of the indicated strain. Infective centres were scored after plating on tryptone plates and incubating at 30 °C. ∇ , GW1000 (ref. 6) (parent of fusion strains); Δ , AB1886 (ref. 12) (*uvrA6*); $\circ$, *uvrA215::Mud(Ap, lac)*; $\bullet$, *uvrA215::Mud(Ap, lac)/pDR2000*; $\blacksquare$, *uvrA215::Mud(Ap, lac)/pDR2026*.

We were concerned that as a result of some secondary event, such as a Mu-mediated deletion¹⁰, the Mud(Ap, *lac*) insertion at the *uvrA* locus could have become fused to the regulatory region of some gene other than *uvrA*. We therefore decided to obtain Mud(Ap, *lac*) insertions in the *uvrA* gene without imposing the requirement that the resulting fusions possess specific regulatory characteristics. Thus, we made a search for *uvrA::Mud(Ap, lac)* insertion mutants based on the criterion of UV sensitivity. Colonies containing random insertions of Mud(Ap, *lac*) were replica plated and UV irradiated (10 J m⁻²). Among 110,000 colonies screened, 55 UV sensitive mutants were obtained. Of these, 13 mapped at the *uvrA* locus (~50% linked to *malB* by bacteriophage P1 transduction¹¹). The UV sensitivity of one of these putative *uvrA::Mud(Ap, lac)* isolates is shown in Fig. 1.

Like known excision repair mutants, they showed (1) a marked decrease in their ability to support the growth of UV-irradiated bacteriophage such as λ (host-cell reactivation¹²) (Fig. 1), (2) hypermutability with UV light¹³ (data not shown), (3) wild-type levels of survival and mutagenesis on exposure to the methylating agent methyl methanesulphonate¹⁴ (unpublished results).

To demonstrate conclusively that these isolates were indeed *uvrA* mutants, we carried out complementation tests. The fusion strains were transformed with the plasmids pDR2000 and pDR2026, which are derivatives of pBR322 carrying the *uvrA*⁺ gene and a *uvrA::\gamma\delta* mutation, respectively¹⁵. As shown in Fig. 1, pDR2000 (but not pDR2026) restored wild-type UV resistance and host-cell reactivation levels. Thus, these isolates carry Mud(Ap, *lac*)-generated insertion mutations of *uvrA*.

The original *uvrA::Mud(Ap, lac)* insertion mutant displayed a substantial level of β -galactosidase. The 13 new *uvrA::Mud(Ap, lac)* mutants fell into two classes: eight displayed levels similar to the original strain and five showed very low β -galactosidase levels. The two classes were shown to contain Mud(Ap, *lac*) insertions in opposite orientations by chromosomal mobilization experiments¹¹ (Table 1).

The two classes of *uvrA::Mud(Ap, lac)* mutants were tested for mitomycin C inducibility of β -galactosidase. As expected, β -galactosidase levels in strains in which *lac* was integrated opposite to the direction of transcription were unaffected by mitomycin C. However, the eight isolates containing *lac* oriented in the direction of transcription each showed a stimulation of activity on exposure to the chemical (Fig. 2a). These same strains showed a similar inductive response to UV irradiation and treatment with nalidixic acid. Thus, transcription of the *uvrA* gene seems to be stimulated by agents that either damage DNA or block DNA replication.

The induction of *uvrA* is only one of several bacterial responses to DNA damage. Treatment of cells with a variety of DNA-damaging agents (including those mentioned above) triggers expression of the 'SOS' functions. These include the induction of certain prophages such as λ , the induction of the *recA* protein, and filamentous growth¹⁶; at least some of these 'SOS' responses may be due to increased expression of the set of *din* genes⁶. All these phenomena are coordinately regulated by the *recA* and *lexA* proteins. The *lexA* protein has been identified as the repressor of the *recA* gene¹⁷ (D. W. Mount and J. W. Little; R. Brent and M. Ptashne, personal communication); the *lexA* gene product has a negative regulatory effect on the *lexA* gene itself¹⁸ as well as on *din* genes (C.J.K. and G.C.W., unpublished). The regulatory effects of *recA* seem to be due to the proteolytic activity of the *recA* protein which can cleave both the *lexA* protein¹⁹ and λ repressor²⁰.

We have examined the mechanism by which *recA* and *lexA* act to regulate the *uvrA* gene product. Induction is controlled by the 'SOS'-regulatory circuitry, as the introduction of *recA*⁻ and *lexA*⁻ (uninducible repressor) mutations prevents increased β -galactosidase expression in the fusion strains (Fig. 2a). Furthermore, an allele of *recA* (known as *lexB30*, ref. 21), which specifies a *recA* protein deficient in proteolytic activity but not recombination functions (J. Roberts, personal communication), also reduces the induction (data not shown). This suggests that the *recA* protein is functioning as a protease in the induction process.

If, like *recA* and *lexA*, *uvrA* is directly repressed by the *lexA* protein, then *uvrA::Mud(Ap, lac)* fusion strains lacking the *lexA* gene product should produce high levels of β -galactosidase regardless of whether the *recA* protein is proteolytically active. As shown in Fig. 2b, cells carrying a putative null allele of *lexA* called *spr* (ref. 17) synthesize high levels of β -galactosidase in a

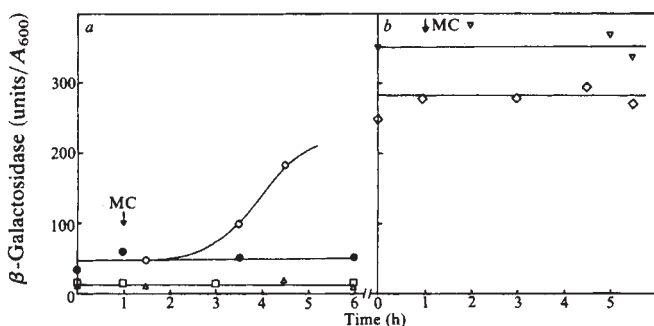


Fig. 2 Kinetics of induction of β -galactosidase in *uvrA::Mud(Ap, lac)* fusion strains. Exponential cultures growing in supplemented M9 media were treated with MC ($1 \mu\text{g ml}^{-1}$) as shown and the specific activity of β -galactosidase determined as described elsewhere⁶. a, $\bullet$, *uvrA215::Mud(Ap, lac)*, no MC; $\circ$, *uvrA215::Mud(Ap, lac)*; $\square$, *recA* *uvrA215::Mud(Ap, lac)*; $\triangle$, *lexA* *uvrA215::Mud(Ap, lac)*; b, ∇ , *spr uvrA215::Mud(Ap, lac)*; $\diamond$, *spr recA* *uvrA215::Mud(Ap, lac)*. The effect of the *uvrA* gene product on induction was determined by introducing pDR2000 into wild-type, *lexA*⁻ and *recA*⁻ cells. No significant differences in induction profiles were observed (data not shown).

Table 1 Orientation of *Mud(Ap, lac)* in representative *uvrA::Mud(Ap, lac)* insertion mutants

Recipient	Pur ⁺ recombination per 10 ⁶ donor cells	
	Donor <i>uvrA215::Mud(Ap, lac)/F_{ts1141lac}</i>	Donor <i>uvrA223::Mud(Ap, lac)/F_{ts1141lac}</i>
<i>purD::Mud(Ap, lac)</i>	510	48
<i>purA::Mud(Ap, lac)</i>	2	1,780

F_{ts1141lac} (ref. 27) was introduced into GW1000 derivatives carrying *uvrA215::Mud(Ap, lac)* (*Lac*⁺, inducible) and *uvrA223::Mud(Ap, lac)* (*Lac*⁻, uninducible). Representative members of the two classes would be expected to transfer host genes in opposite directions when an *F'* *lac* was integrated into the *lac* gene of *Mud(Ap, lac)* to form an Hfr. Cultures of these strains contain a small fraction of Hfrs resulting from *F'* integration into *Mud(Ap, lac)* via *lac* homology. The resulting gradient of transfer of chromosomal markers depends on the orientation of *Mud(Ap, lac)* within *uvrA*. Recipients were derivatives of BW545 (*lac* Δ U169 *rpsL1*) bearing *purA::Mud(Ap, lac)* and *purD::Mud(Ap, lac)* insertions (E. Trefelner and G.C.W. unpublished). (The *purA* and *purD* genes map on either side of *uvrA*.) The presence of *Mud(Ap, lac)* in the recipient prevented killing due to zygotic induction of Mu functions. Equal volumes of exponential cultures were mixed and incubated at 34 °C for 30 min. Matings were interrupted by vortexing and 0.1-ml aliquots were plated on minimal plates lacking adenine and donor nutrients. Plate matings were prevented by shifting the cells to 43 °C after 3 h at 34 °C; only Pur⁺ recombinants escape killing by *Mud(Ap, lac)* *cts* induction. Given the known orientation of *lac* on *F_{ts1141lac}*, we conclude that *uvrA* is transcribed in an anticlockwise direction on the *E. coli* chromosome.

recA⁻ background. Thus, the *lexA* protein may also repress the *uvrA* gene, with induction occurring when the *recA* protein proteolytically inactivates the *lexA* protein in response to DNA damage.

Our suggestions concerning the inducibility of *uvrA* have been recently directly confirmed by Kacinski, Rupp and Seeberg (personal communication), who have found that *uvrA* protein is constitutively expressed at high levels in a *spr recA* strain, and that synthesis of *uvrA* protein is induced by nalidixic acid, a treatment which induces SOS functions.

During our search for UV-sensitive mutants, we isolated two putative *uvrB::Mud(Ap, lac)* fusions. These (1) map at the *uvrB* locus (20% co-transducible with *gal* by bacteriophage P1 transduction), (2) give rise to the same set of excision repair-deficient phenotypes as do the *uvrA* insertions, and (3) synthesize β -galactosidase. Although, curiously, these fusions show little mitomycin C inducibility, they display levels of UV and nalidixic acid inducibility comparable with the *uvrA* fusion strains (data not shown).

The findings that components of the excision repair system are synthesized at increased levels in response to DNA damage imply that *E. coli* induces certain repair capabilities as part of its overall defence strategy. The inducibility of excision repair components helps explain certain earlier observations. First, inactivation of the *lexA* protein (which results in the induction of the *uvrA* gene product) increases the UV resistance of *recA* mutants; this increased resistance is dependent on a *uvrA*⁺ genotype²². Second, *recF* and *umuC* mutants show decreased levels of inducible (Weigle¹⁶) reactivation of UV-irradiated bacteriophage; in each case a *uvrA* mutation eliminates the remaining activity^{23,24}.

The specific mechanism by which induction of the *uvrA* gene product enhances cell survival is unclear. In conditions in which *uvrA*⁺-dependent inducible UV resistance is observed, there seems to be no increase in the levels of 'short patch' excision repair²⁵. Thus, these proteins may be functioning in another repair process. One such possibility is another type of excision

repair called 'long patch' repair²⁶. Like the induction of the *uvrA* gene product, long patch repair is *recA*⁺*lexA*⁺ dependent.

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Binding of an SV40 T antigen-related protein to the DNA of SV40 regulatory mutants

R. McKay

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA

D. DiMaio

Department of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA

When simian virus 40 (SV40) infects host cells, the viral genes are expressed in two phases. Early in infection, the virus genome is transcribed and translated to yield two related proteins, 'large T' and 'small t' antigens. Later the rate of synthesis of the early messenger RNA falls and the viral genes coding for the coat proteins are transcribed and translated. Early gene transcription is inhibited only when an active large T antigen is present in the cell (for review see ref. 1). These and other observations have led to the hypothesis that T antigen acts as a negative feedback repressor of its own RNA transcript^{2–4}. The properties of SV40 tsA mutants, which produce temperature-sensitive T antigen, provide evidence for another role of T antigen—in the initiation of viral DNA replication⁵. Mutations around the origin of DNA replication in SV40 lead to a *cis*-acting defect in DNA replication^{6,7}. These mutations fall outside the region of the A gene which codes for T antigen but within the region known to bind T antigen⁸ and the closely related and more easily prepared D2T protein^{9,10} which is synthesized by an adeno-SV40 hybrid virus Ad2D2 and seems to differ from SV40 T antigen only in its amino-terminal segment¹¹. Revertants of origin-defective mutants have been isolated with second site mutations within the DNA A gene coding sequence¹². These data support the view that T antigen modulates transcription and replication of SV40 DNA by binding to one or more sites in and around the viral origin of replication. We present here evidence to show that mutants of SV40 carrying constructed mutations in the regulatory region¹³ show reduced binding of D2T protein *in vitro*. This is the first demonstration in a eukaryote that a mutation in a regulatory DNA sequence alters the binding of a controlling protein *in vitro*.

Figure 1 shows the organization of the relevant part of the regulatory region in SV40. There are apparently three tandem

D2T protein binding sites^{9,10} spanning a segment known to be involved in the control of DNA replication^{6,7,13,14}. The 5' termini of the early mRNA and some of the late mRNAs also fall in this region of the DNA^{15,16}. The sequence of this segment of wild-type DNA and of the three mutant DNAs used in this study are shown in Fig. 1. The mutants cs (cold-sensitive for plaque formation) 1088 and 1085 were constructed by site-specific mutagenesis¹³; their altered sequences fall within the region defined by DNase and methylation protection studies as the first D2T protein binding site^{9,10}. By contrast, the mutant 812 lacks the second and third binding sites but the first site is unaltered⁷. These changes in sequence around the origin of replication allow distinction of the contribution of different parts of the regulatory region to T antigen binding.

An immunoassay for DNA-protein interaction has recently been developed¹⁷. An important feature of immunoassay procedures to study hormone-receptor interactions is that quantitative measurements of affinity can be made in the presence of complex protein mixtures. The ability to manipulate DNA suggests that DNA fragments of defined sequence can be used, like hormones, as probes for high-affinity DNA binding proteins in complex cell extracts. Assays of this sort are simple to perform, can be used to screen many samples such as mutant viruses and may be particularly valuable in studying the specific binding of regulatory proteins to chromatin rather than DNA. The defective adenovirus Ad2D2 synthesizes a protein related to SV40 T antigen¹¹. This protein, D2T, is known to bind preferentially to DNA sequences around the SV40 origin of replication¹⁰. Using a monoclonal antibody (RMT-1) specific for SV40 T antigen, DNA fragments containing the regulatory sequences around the wild-type replication origin are preferentially precipitated. The controls in this experiment are of two types: first, extracts of HeLa cells infected with adenovirus Ad2⁺ alone show no preferential precipitation of DNA fragments containing the SV40 origin. Second, SV40 DNA fragments lacking the origin of replication are only immunoprecipitated by partially purified D2T when the protein is present in excess and all the origin-containing fragments are already bound by D2T. The immunoassay was used to obtain both kinetic and equilibrium measurements of the affinity of DNA-protein interaction. Both the half life of the complex once formed and a Scatchard equilibrium treatment gave data consistent with an equilibrium dissociation constant of 10^{–12} M. Similar values have been obtained with the nitrocellulose filter binding assay¹⁸.

Site-specific mutagenesis in the regulatory region of SV40 gives viruses with altered properties. The explanation proposed for this alteration in phenotype is that the interaction of T antigen with DNA is changed in the mutant virus. Consistent

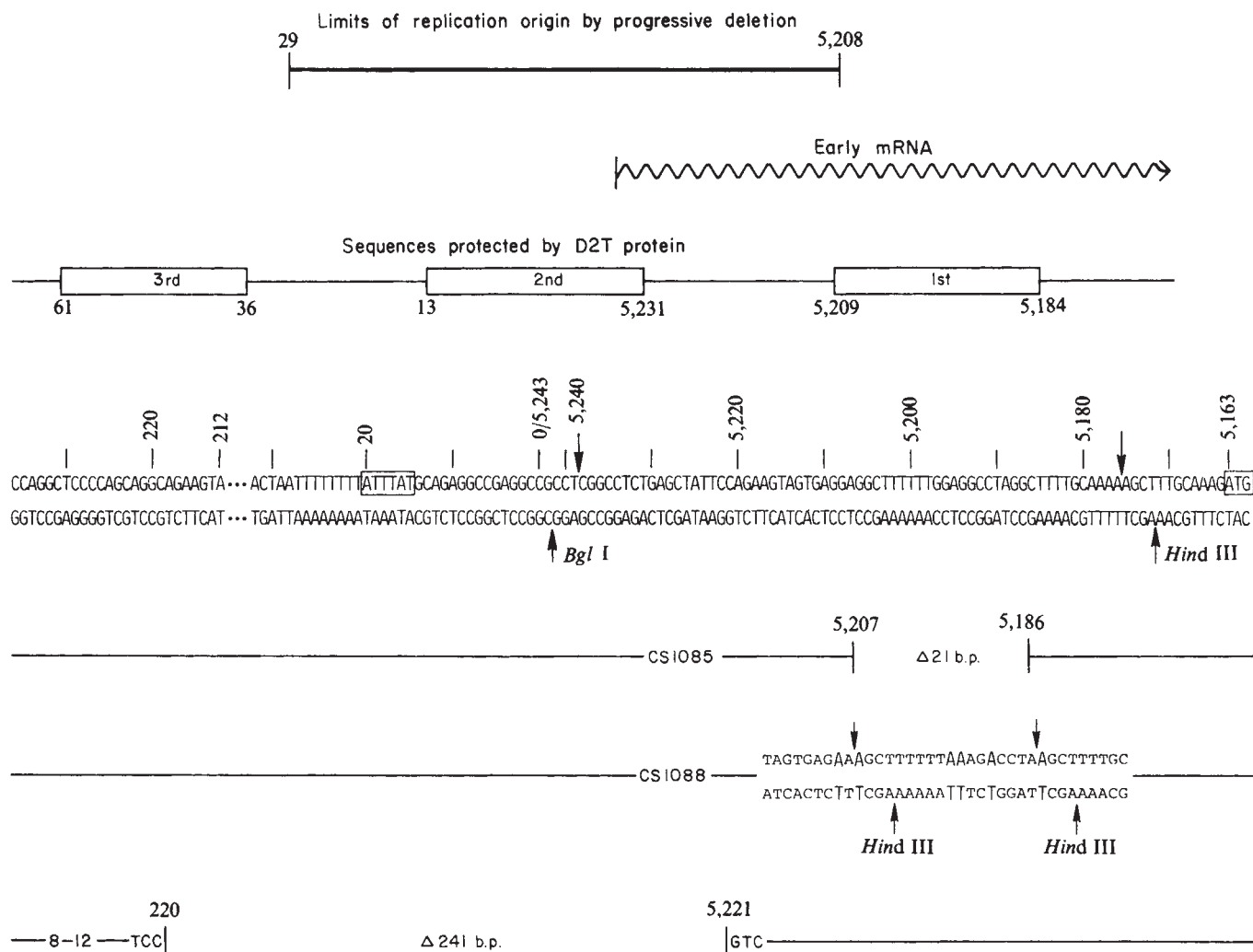


Fig. 1 The structure of the regulatory region of SV40. The wild-type DNA sequence around the origin of SV40 replication is shown in the middle of the figure (numbered according to the BB convention¹). Arrows indicate cleavage sites for the restriction endonucleases *Hind* III and *Bgl* I. The initiation codon for large T and small t antigens and the ATTTAT box are enclosed on the early sense strand. Above this are shown the sequences protected by D2T from methylation and DNase I digestion^{9,10}, the 5' end and direction of transcription of early mRNA¹⁶ and the limits of the replication origin^{13,14}. Below the wild-type sequence the structure of mutant DNAs used in this study are shown^{7,13}. The base substitutions generated in cs 1088 by bisulphite treatment are shown in larger type. Note the two new *Hind* III sites in this mutant. b.p., Base pairs.

with this view, second-site revertants to wild-type phenotype have been mapped to the structural sequences coding for SV40 large T antigen¹². Using a series of deletions around the origin the sequences responsible for the preferential immunoprecipitation of the regulatory region have been mapped within the limits of base pair 5 to base pair 5,189 (ref. 17). Here we have studied the specific D2T binding to virus genomes carrying less drastic alterations in their regulatory region. Our results show directly that these mutant viruses are altered in their ability to interact with a regulatory DNA binding protein.

When SV40 DNA is cut with the enzyme *Bst*NI (an isoschizomer of *Eco*RII, ref. 19), the origin of replication and the D2T protein binding sites are contained in the 311-base pair G fragment. After incubation of ³²P-labelled, *Bst*NI-digested wild-type SV40 DNA with D2T protein followed by immunoprecipitation with anti-D2T antibody, this fragment is preferentially recovered (Fig. 2). By contrast, *Bst*NI G fragments derived from cs 1088 and cs 1985 DNA and carrying mutations in the first binding site show a greatly reduced level of specific binding (Fig. 2). As the *Bst*NI G fragment of cs 1085 (containing a deletion of 21 base pairs) can be resolved from the wild-type G fragment by gel electrophoresis, mixtures of wild-type and

mutant fragments could be tested for relative binding of D2T protein. The result confirms that D2T protein binds the mutant fragment markedly less than the wild-type fragment. As noted in Fig. 2 legend, the binding assays were carried out at a concentration of 20mM NaCl. At high concentrations of NaCl specific binding was inhibited, presumably due to inhibition of ionic interactions between D2T protein and the binding site, but the differential binding of mutant and wild-type G fragment was apparent at all salt concentrations tested.

The binding of the origin-containing DNA restriction fragment was measured relative to another restriction fragment. The autoradiograph shown in Fig. 2 was analysed using a computerized scanning system with four elements: (1) Optronix P1000 scanner; (2) PDP 11/60 computer; (3) Grinnell graphics system, and (4) the QUEST software system (J. Garrels, Cold Spring Harbor Laboratory). The ratio of the integrated intensity of the *Eco*RII/G fragments was 6.5 (s.d. $\pm$ 1.3) for wild-type DNA, 1.87 (s.d. $\pm$ 0.44) for the mutant cs 1085 and 0.81 (s.d. $\pm$ 0.25) for the mutant cs 1088. Note that the *Eco*RII/C fragment is 673 base pairs long and the *Eco* RII/G fragment only 311 base pairs, thus the relative molar binding of wild-type RII/G to RII/C fragments is 13.3. These figures clearly show that

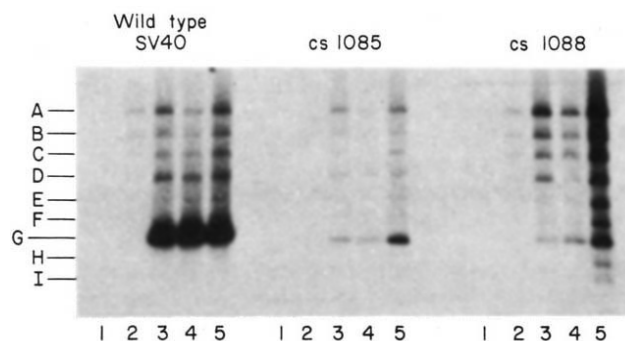


Fig. 2 Immunoprecipitation of SV40 DNA restriction fragments bound by D2T protein. 5 ng of *Bst*NI-digested SV40 or mutant DNA (specific activity 5×10^7 c.p.m. μg^{-1}) were incubated with increasing amounts of partially purified D2T protein: 1, 0 μl ; 2, 25 μl ; 3, 100 μl ; 4, 200 μl ; 5, 300 μl , in 20 mM sodium chloride, 2.0 mM dithiothreitol (DTT), 0.1% bovine serum albumin, 0.1 mM EDTA, 0.05% NP40, 40 $\mu\text{g ml}^{-1}$ phenylmethylsulphonyl fluoride, 3% dimethyl sulphoxide, for 10 min at 25 °C. All the experiments reported in this paper were carried out with the same partially pure preparation of the D2T protein. The bound DNA fragments were then immunoprecipitated by addition of 30 μl of monoclonal anti-D2T antibody for 10 min, followed by 20 μl of rabbit anti-mouse immunoglobulin and 60 μl of fixed *Staphylococcus aureus*. The detailed characterization of this assay is described elsewhere¹⁷. The immunoprecipitated fragments were released from the fixed bacteria with 1% SDS in 10 mM Tris-HCl pH 8.0/1 mM EDTA and analysed by agarose gel electrophoresis and autoradiography. Fragment G contains the replication origin and adjacent sequences. The D2T protein used in this assay was partially purified from HeLa cells infected with Ad2⁺D2 virus (50 plaque-forming units per cell) and grown at 37 °C for 30 h. Nuclei were prepared by hypotonic lysis and the nuclear D2T protein extracted in buffer A (0.15 M NaCl, 10 mM Tris-HCl pH 8.0, 1 mM EDTA, 0.5 mM DTT). This crude nuclear fraction was further purified by chromatography on DE52 (elution in 0.25 M NaCl, 10 mM Tris-HCl pH 8.0, 1 mM EDTA, 0.5 mM DTT). This fraction was then precipitated with 50% ammonium sulphate, dissolved in buffer A plus 30% glycerol and stored at -70 °C. Polyacrylamide gel electrophoresis showed D2T to be one of four major proteins present at this stage.

quantitative scanning of the autoradiograph in Fig. 2 confirms that mutations in the first D2T binding site substantially reduce the D2T-mediated precipitation of these DNA fragments.

In Figure 3 the binding of the *Hind*III fragments of wild-type SV40, cs 1088 and 812 are compared. The base substitution in cs 1088 generate two new *Hind*III restriction sites close to the start of the second D2T protein binding segment¹³. These new restriction sites allow the preparation of a DNA fragment containing only binding segments 2 and 3; as seen in Fig. 3 this fragment shows only very slight preferential binding to D2T protein. In contrast, the wild-type and 812 *Hind*III fragments bind D2T protein strongly. Note that in the case of mutant 812 this strong binding occurs in the absence of the second and third sites. We can conclude that the first site alone can bind D2T protein tightly, but the second and third binding sites are not strong independent sites.

Our main conclusion from these studies is that mutations in a regulatory segment of the SV40 genome, outside structural gene sequences, affect the interaction of this segment of viral DNA with a regulatory protein. Mutations located in the previously defined first D2T protein binding site within the regulatory segment^{9,10} cause a marked decrease in binding of the D2T protein. (Recently we have found that these mutations also cause diminished binding of T antigen partially purified from SV40-infected monkey cells.) The decreased binding is not related to the use of monoclonal antibody in our assay, as serum from hamsters bearing SV40-induced tumours served equally

well. In contrast, mutations in the second T antigen binding site had little or no effect on binding of D2T protein (Fig. 3 and ref. 17). Thus it seems that D2T protein can bind independently to the first site, but poorly or not at all to the isolated second plus third sites. Testing of other mutants with base pair substitutions in this region should help determine which bases contribute most to the specific interaction with D2T protein and with T antigen.

The cold-sensitive regulatory mutants used in this study form small plaques at 32 °C but wild-type plaques at 37 and 40 °C (ref. 13). They therefore seem to have a defect in virus growth at the lower temperature, previously postulated to be due to cold-sensitive interaction of a regulatory protein with an altered signal in the viral DNA¹³. We attempted to demonstrate a differential effect of temperature on the binding of D2T protein to mutant DNA fragments, but due to greater non-specific binding at elevated temperatures, a clear effect was not found.

Regarding the possible physiological significance of the interaction of T antigen in the first binding site of the SV40 regulatory segment, an attractive hypothesis is that this interaction is responsible for inhibiting transcription of the gene for T

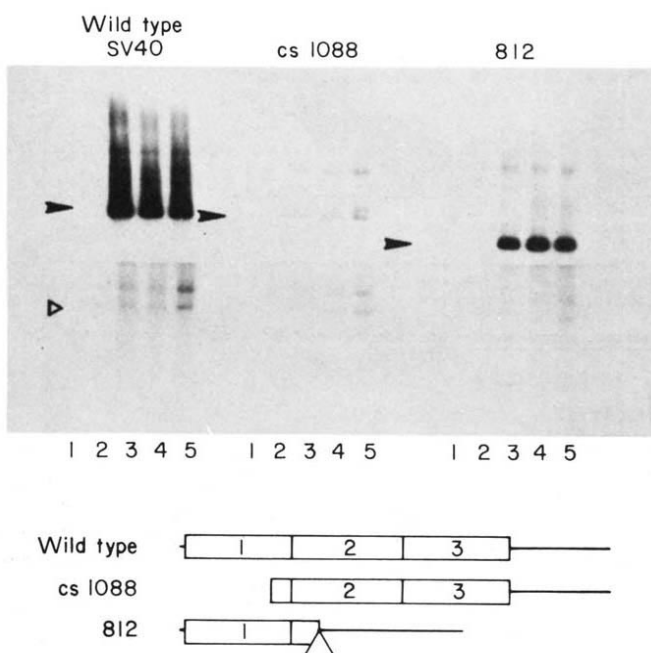


Fig. 3 Immunoprecipitation of *Hind*III DNA fragments containing different combinations of the three D2T protein binding sites. The structure of the *Hind*III fragments spanning the origin is shown in the lower half of the figure with D2T binding sites represented by numbered boxes. The wild-type *Hind*III fragment starts close to the first site and spans all three sites. The *Hind*III fragment from deletion mutant 812 contains only an intact first site. The *Hind*III fragment derived from cs 1088 contains only sites 2 and 3. The D2T binding properties of these fragments were assayed as described in Fig. 2, using 100 μl of D2T per point. The solid arrowheads indicate the position of the origin-containing fragments after immunoprecipitation and electrophoresis; the open arrowhead shows a smaller *Hind*III restriction fragment of SV40 used as an internal control in computerized scanning of the autoradiograph. The lower half of the figure has been overexposed to show the control fragments. Integrated intensity in the origin fragment relative to the control fragment was 10.75 for wild-type DNA, 4.39 for the mutant cs 1085 and 8.79 for the deletion mutant 812 which lacks the second and third binding sites. The high binding of fragments larger than the origin-containing fragment of wild-type SV40 DNA is caused by a small proportion of partial restriction fragments. These immunoprecipitated partial fragments were not included in the relative binding figures given.

antigen. Consistent with this possibility are the presently known properties of mutants cs 1085 and cs 1088, that is, overproduction of early viral RNA and T antigen, and nearly normal rates of viral DNA replication (D. D. and D. Nathans, unpublished observations). More detailed studies of these and similar mutants, including *in vitro* transcription of their DNAs, will be required to test this hypothesis fully.

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Network structure in gels of rod-like polypeptides

Kohji Tohyama & Wilmer G. Miller

Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455, USA

The formation of a mechanically self-supporting, macromolecular gel or network is understood on a molecular scale in terms of cross-links or branch points, either of a permanent nature by covalent bond formation, or of a reversible nature, such as in the gelation of gelatin. In thermally reversible gels each branch point may contain numerous monomeric units, indicative of local crystallite or aggregate formation, as in cellulose acetate¹ or gelatin^{2–4}, or only two monomeric units as in polyacrylylglycinamide⁵. The formation of reversible networks in nonionic, rod-like polypeptide homopolymers^{6–8}, particularly at low concentrations (<0.1 wt%), is surprising in that the molecular origin of the branch points is not obvious. We have suggested previously⁸ that this network constitutes a thermodynamic phase resulting from a particular kinetic mechanism of phase formation made favourable by the unusual polymer-diluent phase equilibria occurring with stiff-chain polymers. Here, the network is visualized by electron microscopy, and shown to be compatible with the proposed mechanism.

Examples of gels visualized by microscopy are shown in Fig. 1. Typically, an isotropic solution was cooled to the desired gelation temperature, held overnight, quenched in Freon 22 cooled with liquid nitrogen, and at -150°C fractured, etched, shadowed (Pt–C) and replicated with a thick carbon layer. The

replica, after removal of polymeric material, was viewed in a Zeiss 9 S-2 electron microscope. Polybenzylglutamate (MW 200,000) networks generally appeared random (Fig. 1a); occasionally regions seemed to have been under uniaxial deformation when quenched (Fig. 1b). Virtually all strands begin and end at branch points.

A freshly fractured surface was shadowed without etching. A typical micrograph (Fig. 1c) shows many ends and spurs, as one might expect with a fractured network. Occasionally a fracture-knife gouge across a network strand was clearly visible. Furthermore, micrographs from isotropic solutions cooled rapidly through the gelation region by quenching in liquid nitrogen showed no network structure. These results indicate that the visualization of the polypeptide networks was not an artefact of sample preparation.

For comparison a network formed from a more flexible polymer was visualized—a gelatin network (Fig. 1d). The micrograph is quite distinct from the polypeptide micrographs, but similar to micrographs of agar and acrylamide gels prepared in a similar manner^{9,10}. Micrographs of other gels have been obtained using different preparation techniques^{2,11,12}. However, we show elsewhere¹³ that our gelatin micrograph is an artefact of sample preparation.

The micrographs of the polypeptide networks reveal strands or fibres with cross-links that do not result from the contact of nonaligned fibres, that is, the cross-links/branch points tend not to have four arms. The fibres are of diameters ranging from hundreds to more than a thousand angstroms, and are composed of bundles of aligned rods (rod diameter $\sim 20\text{\AA}$). The spacing between fibres seems to be typically of the order of $1\text{ }\mu\text{m}$. These values are not certain as the platinum may shadow polymer and solvent differently in the unetched samples, and a depth scale is lacking in the etched samples. However, etched and unetched samples do give similar results.

We believe that the origin and structure of these networks may be the result of the kinetics of phase separation. Typically, when a solution is brought from a uniphase into a biphasic region the new phase forms by a nucleation and growth mechanism. However, if the solution is brought into a region of thermodynamic instability ($\sigma^2 G/\sigma c^2 < 0$ where G = Gibbs free energy and c = concentration) rather than metastability, the spinodal decomposition theory of Cahn and Hilliard¹⁴ predicts phase separation by a non-nucleation mechanism, whereby the spatial variation in solution composition is continuous with a characteristic periodicity. Material flows spontaneously against a concentration gradient. Early in the phase separation each phase is predicted to be continuous in the other phase. In systems which flow, ageing occurs by tube flow¹⁵, leading eventually to the breakup of the network-like morphology. Most aspects of the theory have been tested experimentally^{15–21}.

Application to the polypeptide gels is made by reference to the Flory phase diagram for rigid rod polymers (Fig. 2) which corresponds closely to that observed for polybenzylglutamate^{7,22}. A network forms when a solution is cooled and crosses into the wide biphasic region (designated 'Network'). The very drastic polymer-solvent unmixing which occurs with only a slight change in polymer-solvent interaction should allow entry into the unstable region even at low concentrations. Thus, if phase separation occurs by spinodal decomposition, the network which forms is simply the polymer-rich phase, a damp fibre, which is bicontinuous with the solvent-rich phase. The polymer-rich phase has no tendency to flow, which accounts for the persistence of the network after phase separation is complete. The mean periodicity in concentration fluctuation can be predicted^{18,19,23}, which should correspond to the interfibre spacing. Fibre thickness may then be calculated by mass balance. Application to our samples predicts a spacing of the order of $1\text{ }\mu\text{m}$ and a mean fibre thickness of several hundred angstroms²³, which agree with our observed values.

Other rod-like synthetic polymers form gels²⁰. Also, many biological macromolecules form rod-like assemblies and gels, which sometimes affect cell morphology, for example, sickle-cell

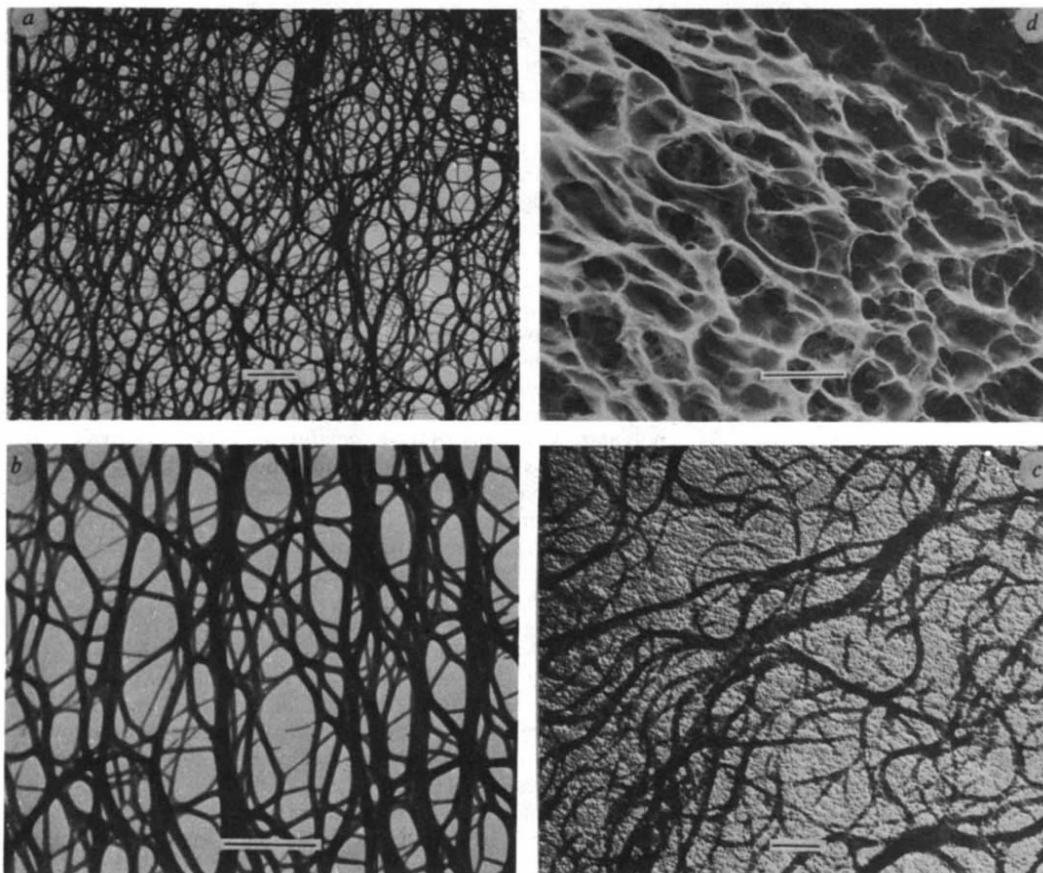


Fig. 1 Electron micrographs of 1 wt% polybenzylglutamate gels (a–c) and of a 3 wt% gelatin gel (d). Scale bar 1 μ m. Etch times: 10 s (a, b), 0 s (c), 60 s (d).

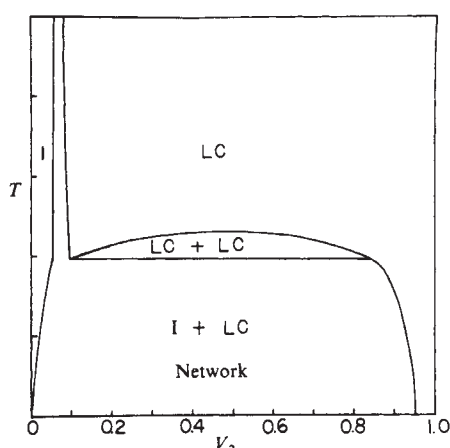


Fig. 2 Flory²² temperature–composition (volume fraction polymer) phase diagram for rigid rod–diluent (rod axial ratio 150), which corresponds closely to that observed for polybenzylglutamate⁷. A network (gel) forms when a solution is cooled and crosses into the wide biphasic region (Network). The phases present are isotropic (I) and liquid crystalline (ordered) (LC).

haemoglobin, actin, tubulin and tobacco mosaic virus^{20,24–26}. Thus the mechanism by which a rod-like polymer forms a network spanning the confining vessel may be of interest in understanding passive structure formation in more complex systems.

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Structure of the protein and DNA in fd filamentous bacterial virus

D. W. Banner, C. Nave & D. A. Marvin

European Molecular Biology Laboratory, Postfach 10.2209, 6900 Heidelberg, FRG

The virion of filamentous bacterial viruses comprises a cylindrical protein shell of o.d. ~60 Å and i.d. 20 Å, containing a single-stranded circular DNA molecule which has two oppositely directed but not base-paired strands extending the length of the virion. The assembly of the virion involves an intracellular prepackaging of the DNA with a viral DNA-binding protein which is then displaced by the coat protein as the

growing virion crosses the bacterial membrane¹. Studies of the virion by X-ray fibre diffraction show that the protein coat consists largely of α -helices oriented roughly parallel to the axis of the virion^{2,3}. As the normal to a planar peptide tends to align normal to a magnetic field, it is possible to improve significantly the orientation of virions in fibres using a strong magnet^{4,5}. The success of this technique with the Pf1 strain of virus led us to apply it to the better-known fd (f1, M13) strain. We report here new information about the arrangement of protein and DNA in the fd virion obtained from the improved diffraction pattern (Fig. 1).

The fd virion is $\sim 8,700$ Å long; its DNA contains 6,408 nucleotides, and its coat contains $\sim 2,760$ 50-residue protein molecules. The distribution of intensity on the fd fibre patterns indicates that rods of electron density slope from large to small radius and also slew radially, as proposed⁶ for the similar virus Pf1. The position of layer lines on the diffraction patterns gives information about the relative positions of the protein subunits. There is a set of layer lines at orders of 32 Å, and a set of meridional reflections at orders of 16 Å. Calculation of mass per length (as described in Table 9 of ref. 7) using accurate molecular weight data derived from the DNA sequence⁸, shows that in the 16-Å repeat distance there are 5.1 protein subunits ($\pm 7\%$) and 11.8 nucleotides ($\pm 4\%$). These numbers imply 2.72 Å average axial separation between nucleotides in each of the two DNA strands.

The meridional reflection at 16 Å was previously interpreted³ as being due to a periodic perturbation of a helical arrangement of subunits, with a repeat of the perturbation every five subunits. This perturbation predicts satellite layer lines which could not be seen on poorly oriented patterns, but their possible presence

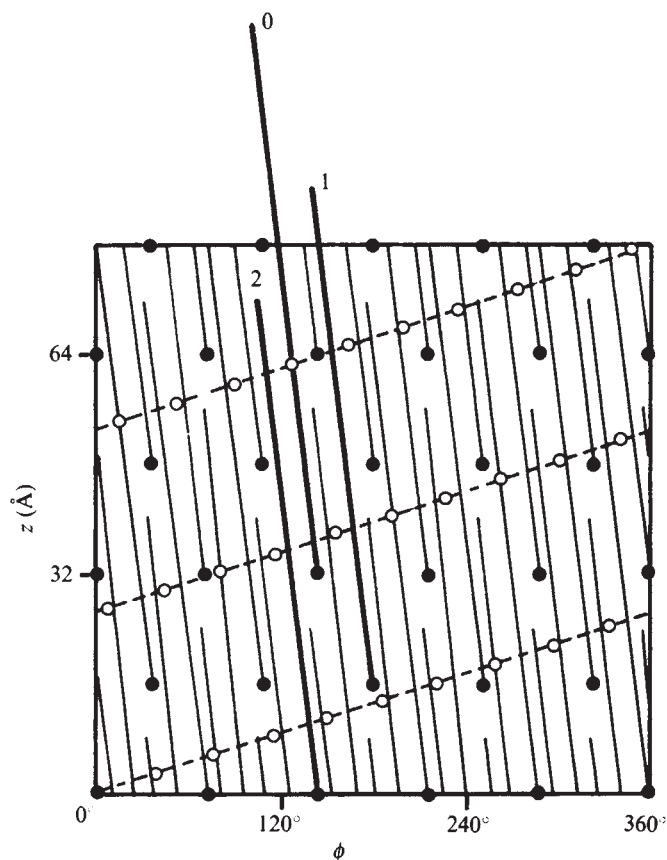


Fig. 2 Surface lattice of the fd virion. The surface lattice is generated by projecting equivalent points along radii onto a cylinder of 13 Å radius, and then cutting the cylinder along a vertical line to give a 2-dimensional representation. ●, Projections of equivalent points in each protein subunit, and the solid lines represent the projections of the α -helix axes. The bold solid lines indicate the three nearest neighbours shown in Fig. 3. ○, Projections of equivalent points in each DNA nucleotide pair, and the broken line represents the projection of one strand of the DNA helix. The fact that the DNA helix passes through surface lattice points of the protein at a (ϕ, z) of $(0, 0)$, $(72^\circ, 32 \text{ Å})$, and $(144^\circ, 64 \text{ Å})$ may be fortuitous, but note that a nucleotide/protein ratio of 2.30 would give identical DNA-protein environments every 23 nucleotides along one DNA strand (every 64 Å along the axis), and a nucleotide/protein ratio of 2.40 would give identical DNA-protein environments every 12 nucleotides (every 32 Å). The relative phase of the DNA and protein is unknown; that is, the DNA helix could be shifted in ϕ and/or z relative to the protein helix. The line-group symmetry¹⁴ of the protein assembly is sr , $N = 5$.

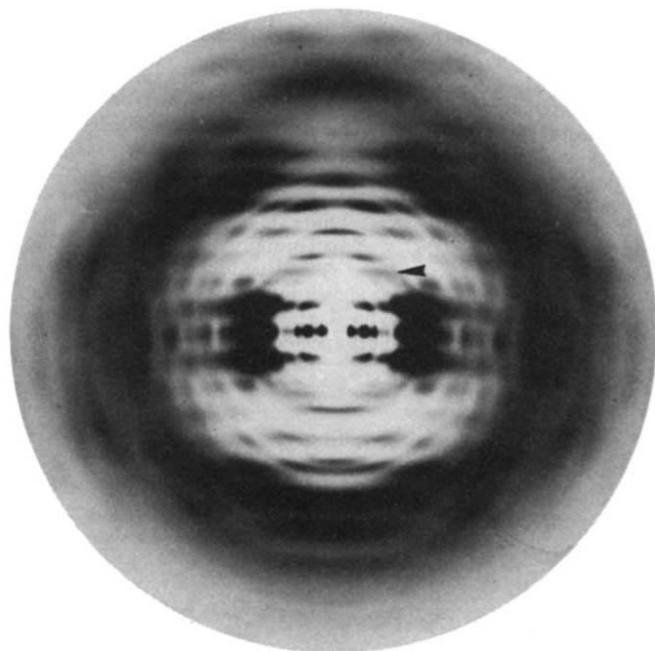


Fig. 1 X-ray fibre diffraction pattern of fd filamentous virus. Fibre axis vertical. Preparation of virus and X-ray fibre diffraction techniques are described elsewhere³. In the purified virus solution, $>98\%$ of the protein migrated with the major coat protein and $>95\%$ of the DNA migrated with viral DNA in appropriate gel electrophoreses. The virus solution was titrated to pH 2 (below the isoelectric point) before preparing fibres, because fibres prepared from virus solutions at neutral pH give double orientation (A. Fowler, personal communication). Protein layer lines occur at orders of $c = 32.3$ Å with meridional reflections on even layer lines. DNA layer lines occur at 26.7, 13.35 (arrow), 3.42 and 3.02 Å. Unit cell parameter $a = 60.7$ Å; 92% relative humidity; specimen-film distance 4.20 cm; tilt 16.6° ; standard deviation of disorientation 3.5° . Fibre fd 45.2; film 5148. $\times 1.6$.

could not be ruled out. These satellite layer lines are clearly absent on Fig. 1, therefore such a perturbation is not supported by the data. An alternative interpretation^{9,10} of the 16-Å meridional reflection is that the virion symmetry involves a 5-fold axial rotation axis combined with a 2-fold axial screw axis of pitch 32 Å (Fig. 2). The difference between this kind of arrangement and a perturbed helix is small (see ref. 11, Fig. 10). In particular, subunits with the same shape as in the perturbed helix model pack well (Fig. 3) in the symmetry of Fig. 2, and give a calculated diffraction pattern which agrees with the strong 10-Å equatorial and near-equatorial observed data (Fig. 4). The local interactions between nearest neighbours shown in Fig. 3 are similar to those of the structurally related virus Pf1 (ref. 10; Fig. 3) at 7 Å resolution.

The diffraction patterns of magnetically aligned fd often show faint layer lines at the first two orders of a 26.7-Å periodicity. If

these layer lines are attributed to J_1 and J_2 Bessel functions respectively, the radial positions of the intensity maxima along the layer lines would correspond to electron density at a radius of ~ 8.5 Å. We interpret these layer lines as being due to the pitch of helical viral DNA. DNA of this pitch with 2.72 Å axial separation between nucleotides would then have 9.8 units per turn (Fig. 2). If there is no base pairing, the two strands can be moved relative to one another. We know of no DNA structure of this type, thus we used the approach (see ref. 12) of building molecular models of DNA with these helix parameters and a phosphorus radius of 8.5 Å. (Models with phosphates at a smaller radius¹³ would imply an abnormally short phosphorus-phosphorus distance along an fd DNA strand of 26.7 Å pitch and 2.72 Å axial separation.) The best overall fit to the first three DNA layer lines is given by a model that is unexceptional in the conformation of the sugar-phosphate backbone and the tilt of the bases, but other kinds of models (for example, left-handed or Z-DNA) cannot yet be ruled out.

Meridional intensity at 2.72 Å would be predicted for a regular DNA helix with 2.72 Å axial separation between nucleotides. No meridional or near-meridional intensity is observed at this position, but weak near-meridional intensity is observed on a layer line at 3.02 Å, and strong meridional intensity at 3.42 Å (apparently not consistent with a J_2 Bessel function). These are two of the layer lines predicted for a regular helix of 26.7 Å pitch and 2.72 Å unit rise. These observations, taken together with UV dichroism measurements, would be consistent with a tilted-base conformation of the DNA⁷, except for the fact that intensity directly on the meridian at 3.42 Å is forbidden for these helix parameters. Thus the bases are not all arranged on a simple helix—many are stacked axially about 3.42 Å apart despite the mean rise per nucleotide of 2.72 Å.

The data can be summarized by identifying three separate clusters of layer lines on the diffraction pattern. The layer lines with a 32 -Å repeat are due to coat protein molecules; the layer lines with a 26.7 -Å repeat are due to the DNA which, at low

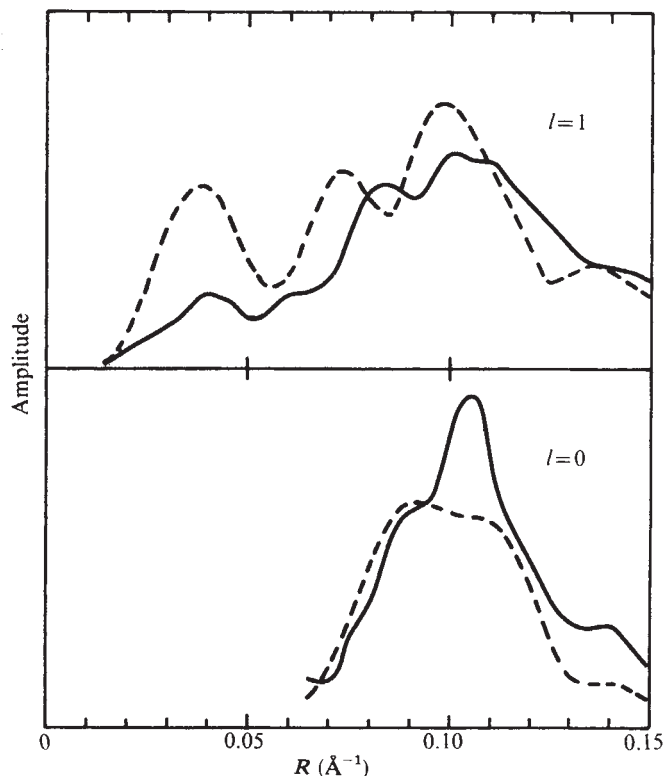


Fig. 4 Distribution of amplitude (absolute values) along layer lines in the strong 10 -Å equatorial and near-equatorial region. Observed amplitudes are represented by solid lines, and calculated amplitudes by broken lines. Intensity data were measured⁶ from the diffraction pattern shown in Fig. 1. The sharp peak at 0.105 Å⁻¹ for $l=0$ is due to crystalline reflections superposed on the continuous transform. The cylindrically averaged Fourier transform was calculated⁶ for the model of Fig. 3. Observed and calculated intensities due to protein were scaled together, using the first seven layer lines, to $R=0.15$ Å⁻¹. The J_0 Bessel function contribution is not shown. The similarity between the intensity distribution on the fd 32 -Å layer lines and the layer lines of Pf1 (ref. 4; Fig. 3) suggests that the coat protein subunit has a similar structure in the two virions. This structure could be either a continuous α -helix rod¹⁰ as shown in Fig. 3, or two α -helix segments occupying roughly the same space in the virion as the continuous rod, but connected in a different way.

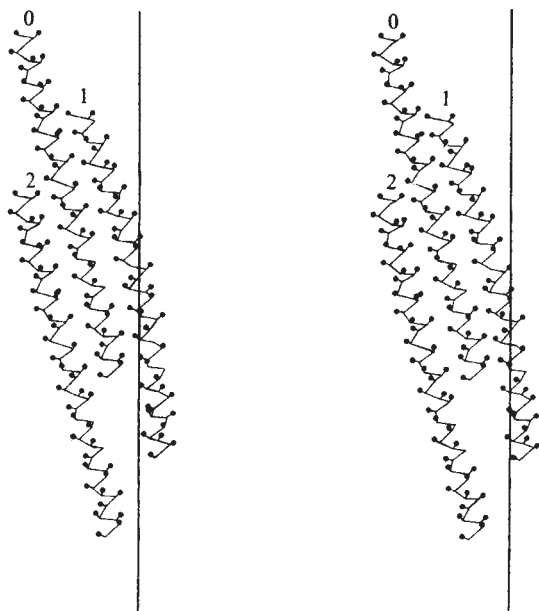


Fig. 3 Stereo pairs showing the interactions between an α -helical subunit and its nearest neighbours in a model of the fd virion fitting the data to 7 Å resolution. The lines connect α -carbons along the sequence. The points represent positions of β -carbon atoms. The view is perpendicular to the virion axis (vertical line) with the N-terminus upwards. These three molecules correspond to the bold lines in Fig. 2.

resolution, follows a helix of 26.7 Å pitch and 8.5 Å radius; and the 3.42 Å meridional reflection is indicative of base stacking but is not consistent with a regular helix of 2.72 Å rise per nucleotide.

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Structure of TANDEM and its implication for bifunctional intercalation into DNA

M. A. Viswamitra*, Olga Kennard†, William B. T. Cruse†, Ernst Egert†, George M. Sheldrick‡, Peter G. Jones‡, Michael J. Waring§, Larry P. G. Wakelin§ & Richard K. Olsen||

* Indian Institute of Science, Bangalore, India

† University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK

‡ Anorganisch-Chemisches Institut, University of Göttingen, FRG

§ Department of Pharmacology, Medical School, University of Cambridge, Cambridge CB2 2QQ, UK

|| Department of Chemistry and Biochemistry, Utah State University, Logan, Utah 84321, USA

Quinoxaline antibiotics (Fig. 1a, b) form a useful group of compounds for the study of drug–nucleic acid interactions^{1,2}. They consist of a cross-bridged cyclic octadepsipeptide, variously modified, bearing two quinoxaline chromophores. These antibiotics intercalate bifunctionally into DNA^{2,3} probably via the narrow groove, forming a complex in which, most probably, two base pairs are sandwiched between the chromophores^{4,5}. Depending on the nature of their sulphur-containing cross-bridge and modifications to their amino acid side chains, they display characteristic patterns of nucleotide sequence selectivity when binding to DNAs of different base composition and to synthetic polydeoxynucleotides^{4,6,7}. This specificity has been tentatively ascribed to specific hydrogen-bonding interactions between functional groups in the DNA and complementary moieties on the peptide ring^{2,4,5}. Variations in selectivity have been attributed both to changes in the conformation of the peptide backbone⁶ and to modifications of the cross-bridge⁷. These suggestions were made, however, in the absence of firm knowledge about the three-dimensional structure and conformation of the antibiotic molecules. We now report the X-ray structure analysis of the synthetic analogue of the antibiotic triostin A, TANDEM (des-*N*-tetramethyl triostin A) (Fig. 1c), which binds preferentially to alternating adenine–thymine sequences⁷. The X-ray structure provides a starting point for exploring the origin of this specificity and suggests possible models for the binding of other members of the quinoxaline series.

Hydrated crystals of TANDEM, C₄₆H₅₄N₁₂O₁₂S₂·12H₂O, were obtained from a mixture of dimethyl formamide/ethanol/water. Crystal data: orthorhombic, P2₁2₁2₁, *Z* = 4, *a* = 18.400, *b* = 22.126, *c* = 16.435 Å. Diffractometer measurements for 5,422 independent reflections were obtained with MoK α radiation to a resolution of 0.85 Å at 7 $\pm$ 1 °C. The structure was solved with some difficulty by a combination of direct methods and a novel iterative Fourier procedure⁸. Refinement, with all non-hydrogen atoms anisotropic, converged at *R* = 14.7% for 4,111 data with *F* > 2 σ (*F*). Full details will be published elsewhere.

Figure 2 shows the molecular structure of TANDEM. The octadepsipeptide ring is held in a relatively rigid conformation through intramolecular hydrogen bonds NH(L-Val) ... O(L-Ala). The quinoxaline chromophores and the disulphide cross-

bridge project on opposite sides of the peptide ring. The carbonyl groups of L-Cys and L-Val also project from the side opposite to the chromophores. The molecule displays a helical twist and has an approximate 2-fold axis of symmetry. Bond lengths and angles fall within the normal ranges.

The mean planes of the two chromophores are inclined at an angle of 14°. The distance between the Ser α -carbon atoms is 12.2 Å. Assuming that the intercalated complex is of the Fuller–Waring type⁹, with minimal distortion from a classical B-DNA helix, we would expect the chromophore planes to lie parallel, approximately orthogonal to a line joining the Ser α -carbon atoms and separated by a perpendicular distance of 10–11 Å, to allow for bifunctional intercalation¹⁰. These geometrical constraints are readily imposed on the molecule derived from the X-ray analysis by rotations of approximately 20° about the C α –N bonds of D-Ser. Such rotations do not result in steric hindrance with neighbouring groups^{5,11}. The resultant structure (Fig. 3) can be fitted into the narrow groove of a B-DNA helix,

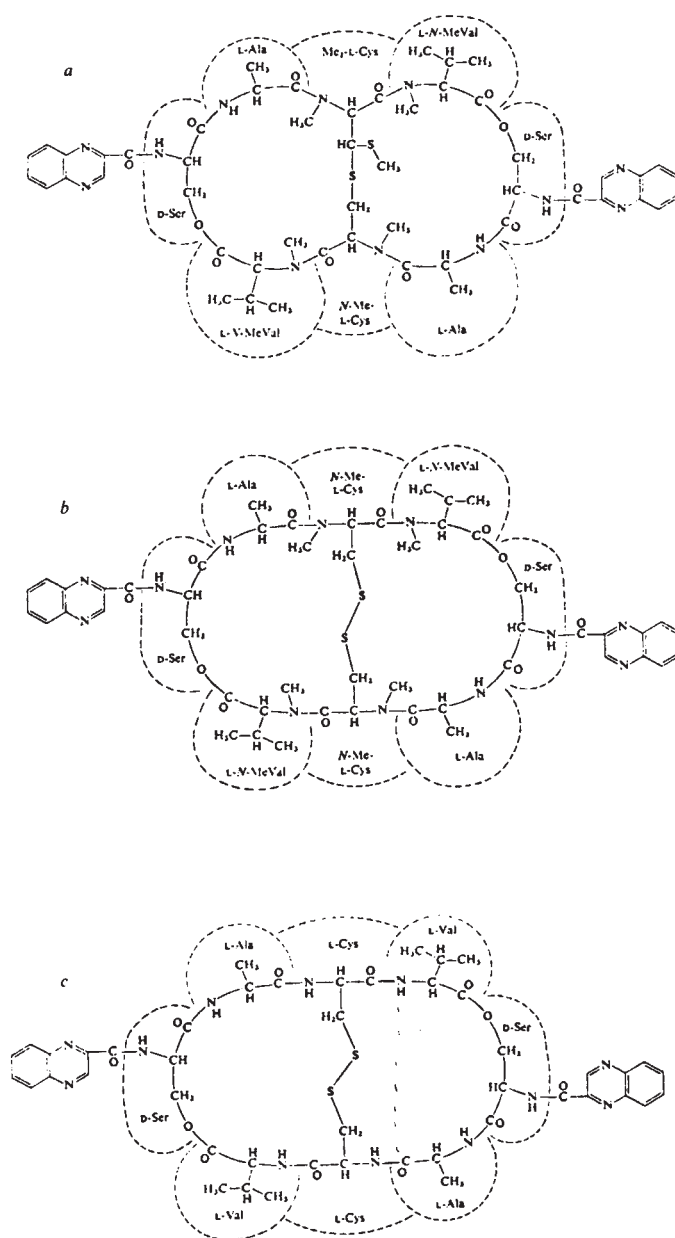


Fig. 1 Structural formulae of echinomycin (quinomycin A) (a), triostin A (b), TANDEM (des-*N*-tetramethyl triostin A) (c).

which is known from solution experiments to be unwound by 45.5° on intercalation of TANDEM^{2,7} (based on an unwinding angle of 26° for ethidium¹²). In this bis-intercalated model the disulphide cross-bridge projects outwards away from the helix, two base pairs are sandwiched between the chromophores and various functional groups of the cyclic peptide are placed in the vicinity of potential hydrogen-bonding sites on the DNA bases. The pseudo-2-fold axis of TANDEM is aligned with the 2-fold axis between the base pairs (Fig. 4).

Binding experiments with DNAs of varying base composition and with synthetic polydeoxynucleotides have revealed that TANDEM binds at least 7,500 times more tightly to poly(dA-dT) than to poly(dG-dC) and there are marked differences in the binding constants for DNAs of different nucleotide composition⁷. This specificity might be explainable in terms of hydrogen

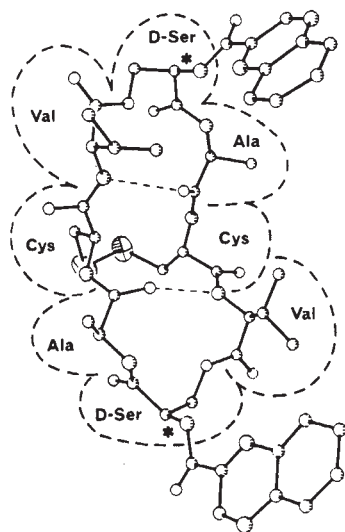


Fig. 2 Molecular structure of TANDEM determined by X-ray analysis at room temperature to a resolution of $0.85 \text{ \AA}$. Dotted lines indicate intramolecular hydrogen bonds between the NH groups of L-Val and the O atoms of L-Ala. These bonds hold the molecule in a relatively stable conformation. Asterisks indicate the N-C $_{\alpha}$ (D-Ser) bonds through which the chromophores are attached to the octapeptide ring.

bonding between the peptide backbone of the drug and the DNA bases, using the model derived from the X-ray structure.

There are two potential hydrogen-bond donors and one potential acceptor in each half of the molecule (Fig. 3) which could be directed towards the interior of the helix on intercalation. The donors are the NH groups of L-Ala and D-Ser and the acceptors are the carbonyl O atoms of L-Ala. The latter are already involved in an intramolecular H bond with the NH group of L-Val (Fig. 2) and in an unfavourable position to accept a second hydrogen bond from the available donors of the DNA base pairs. Of the potential donor groups of TANDEM the NH groups of L-Ala seem to be well placed, as planes parallel to the chromophores oriented as in Fig. 3 and passing through these N atoms are separated by a perpendicular distance of $3.5\text{--}4 \text{ \AA}$. In the intercalated complex, therefore, the NH groups of L-Ala lie co-planar with the sandwiched base pairs. Experiments with CPK models indicate that a reasonable fit can be obtained with hydrogen bonds of $\sim 3 \text{ \AA}$ between the NH groups of L-Ala and the O-2 atoms of thymine in the narrow groove (Fig. 4). In this intercalated model an ApT sequence is sandwiched between the chromophores. The unwinding angle, spread over four base pairs, is $40^\circ\text{--}50^\circ$, consistent with the experimentally determined value for DNA^{2,7}. Attempts to build alternative intercalated

models involving the N-3 atoms of adenine or the NH groups of D-Ser in hydrogen bonding were unsuccessful, nor could we achieve satisfactory intercalation via the major groove. It is known that binding of the related antibiotic, echinomycin, to phage T2DNA is not prevented by the presence of bulky sugar substituents which occlude the wide groove of the helix⁴.

The X-ray structure of TANDEM prompts some suggestions about the character of the intercalation process. Interestingly, the isolated chromophore, quinoxaline-2-carboxamide, unlike simple monofunctional intercalators, shows no detectable interaction with DNA and does not affect the supercoiling of circular DNA^{4,10}. Thus, the driving force for intercalation cannot be ascribed simply to a tendency to form stacking interactions between the chromophores and base pairs. In TANDEM, as shown by the crystal structure, the chromophores are held at the approximate position required for bifunctional intercalation by the relatively rigid depsipeptide backbone, and thus a very important geometrical constraint is already 'locked into' the structure of the ligand molecule. Moreover, all but two of the carbonyl O atoms are directed away from the chromophore side of the drug, where they are best placed to retain their hydrogen-bonding interactions with the surrounding solvent after binding to DNA. Such interactions are apparent in the present crystal structure where all but two of the carbonyl oxygens make at least one hydrogen bond with the surrounding water molecules and there is no direct hydrogen bonding between neighbouring TANDEM molecules. The hydration spheres are even more perfect in a structure of TANDEM determined at -135°C (D. van der Helm, personal communication).

The naturally occurring antibiotics echinomycin and triostin A (Fig. 1a, b) display patterns of selectivity in binding to DNA and synthetic polydeoxynucleotides which, while characteristic for each antibiotic, are quite different from the marked preference of TANDEM for A + T-rich sequences^{2,6,7}. For instance, echinomycin binds well to poly(dG-dC), approximately twice as strongly as it binds to poly(dA-dT), whereas the converse is true of triostin A. These differences from TANDEM are probably

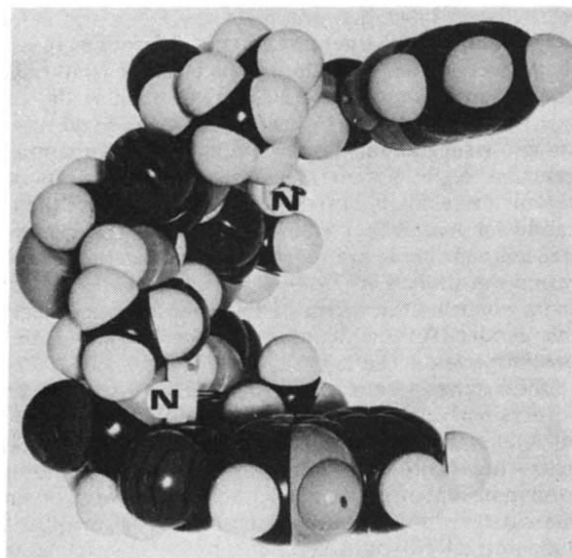


Fig. 3 CPK model of the TANDEM molecule with the quinoxaline chromophores rotated about the N-C $_{\alpha}$ (D-Ser) bonds from the crystal position to allow for bifunctional intercalation. The rotation is about 20° for each chromophore. The nitrogen atoms of L-Ala donating hydrogen bonds to the O-2 atoms of the thymine bases are indicated.

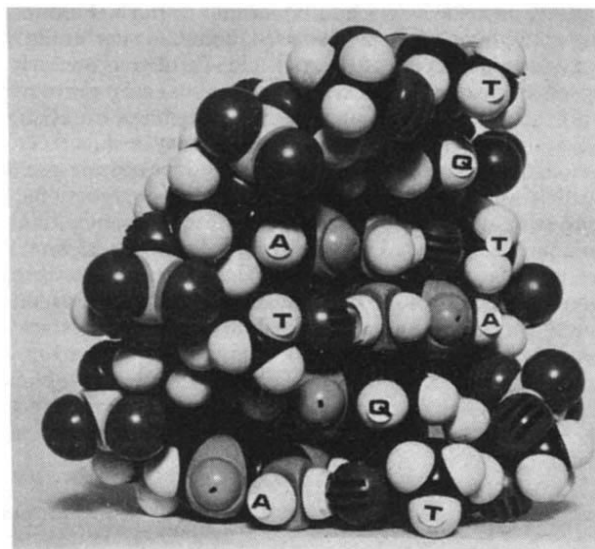


Fig. 4 CPK model of the intercalated complex between TANDEM and a partially unwound B-DNA helix showing the major groove side. The quinoxaline groups (Q) and the bases (adenine, A, and thymine, T) are labelled.

related to the effects of *N*-methylation on the precise conformation of the octapeptide ring and thus the availability of its functional groups for hydrogen bonding^{2,4,5,13}. *N*-methylation of the L-Cys and L-Val residues in the natural antibiotics precludes formation of the intramolecular H bonds observed in TANDEM. Moreover, methylation of the L-Val residues necessarily results in conformational differences which will cause the carbonyl oxygens of L-Ala to project in the same direction as the chromophores. From this position they become available as effective hydrogen-bond acceptors for interaction with donors in the minor groove, that is, the 2-amino groups of guanine nucleotides, which may explain the ability of the antibiotics to bind to sequences containing G.C as well as A.T base pairs.

Extrapolation from the crystal structure of TANDEM to detailed molecular models for quinoxaline antibiotic-DNA complexes must be approached very cautiously. Although various lines of evidence (NMR^{5,13}, conformational calculations^{5,11}) suggest that the peptide backbone in all these compounds adopts a relatively rigid and well defined conformation, there is no experimental evidence that this conformation does not alter on intercalation. Indeed, the significant differences found between the present room-temperature X-ray study and the similar one at -135°C indicate that the orientation of some bonds, particularly those involving carbonyl groups, can be significantly affected by hydrogen bonding to solvent molecules¹⁴. There is also a degree of uncertainty about local DNA conformation^{15,16} and about the orientation of the base pairs with respect to the helix axis in solution¹⁷⁻¹⁹. The recent X-ray study of the daunomycin-hexadeoxynucleotide complex²⁰ has shown that widespread changes can occur in the conformation of the backbone and the positioning of the bases on intercalation. Thus, although the present study gives well defined information about TANDEM itself, only a generalized model can be put forward for the DNA-drug complex. We are continuing our attempts to co-crystallize TANDEM with synthetic A-T deoxyoligonucleotides in the hope of establishing the structure of an intercalated complex directly by X-ray analysis.

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Transglutaminase modifies the carboxy-terminal intracellular region of HLA-A and -B antigens

Jordan S. Pober* & Jack L. Strominger

The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138, USA

The HLA-A and -B antigens, encoded in the major histocompatibility complex, are transmembrane proteins whose carboxy termini project inwards from the cell membrane. The intracellular region of the HLA-B7 antigen is known to include two glutamine residues¹. We have used guinea pig transglutaminase to show that *in vitro* the enzyme couples amines specifically to glutamine residues in the carboxy-terminal region of the HLA-B7 and -A2 antigens. The specificity of this reaction has an immediate practical significance, but may also have a wider biological interest.

HLA-A and -B antigens contain an invariant extracellular polypeptide subunit (β_2 -microglobulin or p12) of molecular weight (MW) 12,000 noncovalently attached to a polymorphic 44,000-MW transmembrane, glycosylated, heavy chain (p44)². The heavy chain has three major regions: a 34,000-MW glycosylated, extracellular amino-terminal segment (p34) that is released from the membrane by papain in a complex with the light chain (p34, 12), a transmembrane hydrophobic segment of about 25 amino acid residues, and an intracellular hydrophilic carboxy-terminal region of about 30 amino acid residues³. Interactions of the HLA-A and -B antigens with intracellular

* Present address: Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts 02115, USA.

constituents must involve the carboxy-terminal intracellular domain.

Transglutaminases are ubiquitous enzymes that catalyse the exchange of the γ -carboxamide ammonia group of a protein glutamine residue within a flexible region of polypeptide for an exogenous primary amine⁴. As shown in Fig. 1, guinea pig liver transglutaminase catalyses the insertion of ³H-putrescine into the heavy chain of HLA-B7 antigen. Similar results were found with HLA-A2 antigen. When dansyl cadaverine was substituted for putrescine, the HLA-B7 or -A2 heavy chain became fluorescently labelled. Neither amine was incorporated into β_2 -microglobulin (the HLA light chain). The location of the probe in the HLA heavy chain was ascertained by limited proteolysis. Papain cleaves native HLA-B7 antigen in detergent solution in a stepwise fashion, releasing first the carboxy-terminal hydrophilic region and then the penultimate hydrophobic region, altering the apparent MW of the heavy chain from 44,000 to 39,000 to 34,000 (ref. 3). As shown in Fig. 2, ³H-putrescine radioactivity is released concomitantly with release of the intracellular carboxy-terminal domain. Thus the glutamine residue(s) that is modified must be one or both of those located within this region. Note that none of the 16 glutamine residues in the extracellular region seems to be modified. Therefore, only the intracellular hydrophilic region contains exposed glutamine residues in a flexible portion of the molecule.

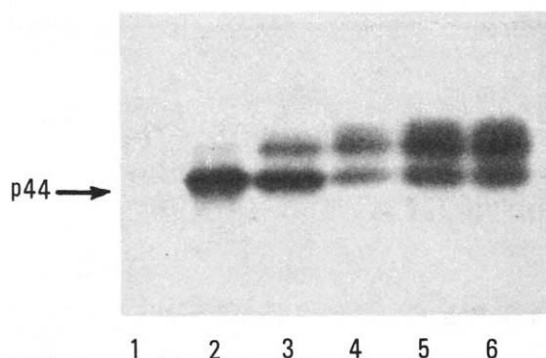


Fig. 1 Transglutaminase modification of HLA-B7. HLA-B7 antigen was purified from the membranes of JY lymphoblastoid cell membranes (doubly homozygous HLA-A2, -B7) by use of allospecific monoclonal antibody immunoaffinity chromatography in a modification (to be published elsewhere) of the procedure of Parham⁹. The pure antigen was adjusted to a concentration of 250 μg protein ml^{-1} in 10 mM Tris-HCl pH 8.0, 0.5% Nonidet P40 (NP40) non-ionic detergent. Guinea pig liver transglutaminase¹⁰ at 50 μg ml^{-1} was pre-equilibrated in a solution made 500 mM in Tris-HCl pH 8.0, 0.5 mM putrescine, 40 mM CaCl_2 and 10 mM dithiothreitol at 37 °C for 30 min. This enzyme mixture (0.5 ml) and 0.5 ml of ³H-putrescine-HCl in H_2O (0.5 mCi, NEN) were added to 1 ml of the HLA-B7 solution and the reaction was allowed to proceed at 37 °C. At different time points, 100- μl aliquots were withdrawn, placed on ice and made 25 mM in EDTA to inhibit the enzyme. Trichloroacetic acid (50 μl of 50%) was added to precipitate the protein. The precipitates were collected by centrifugation 1 h after the last time point was taken and were washed once with acetone to remove excess trichloroacetic acid. Each sample was dissolved in SDS sample buffer, subjected to SDS-polyacrylamide gel electrophoresis on a 20-cm 7–15% linear gradient with the buffer system of Laemmli¹¹, and analysed by Coomassie blue staining (not shown) and by fluorography¹². Lanes 1–6 show the fluorograph of ³H-putrescine radioactivity in the position of the HLA-B7 heavy chain after 30 s, 15, 30, 60, 120 and 180 min of transglutaminase-catalysed modification, respectively. Note that as labelling continues, a portion of the heavy chain shifts its electrophoretic mobility to one of slightly higher molecular weight. The explanation for this shift with time of incubation is unclear.

These results have two additional implications. First, exogenous transglutaminase may be used to modify specifically the carboxy-terminal region of HLA-A and -B antigens for various purposes. For example, spectroscopic probes⁵ may be inserted to study the structure and function of this region of the HLA-A and -B molecules. HLA-A and -B antigens may be fluorescently or radioactively labelled, in a manner that does not perturb the antigenicity of the extracellular portion, for use in functional or binding assays with receptors for these antigens. Transglutaminase may be used as a vectorial label⁶ to assess whether the carboxy-terminal region of the HLA-A and -B antigen is exposed in a reconstituted liposome preparation. Second, these data are consistent with the possibility that intrinsic transglutaminases could be involved in membrane events, including

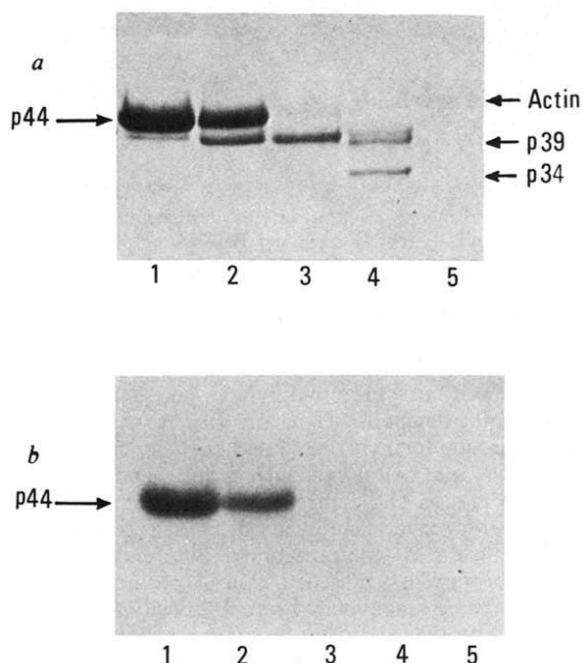


Fig. 2 Limited proteolysis of ³H-putrescine-labelled HLA-B7. HLA-B7 antigen, labelled for 180 min as in Fig. 1 by transglutaminase-catalysed insertion of putrescine into the HLA heavy chain, was subjected to limited proteolysis by papain (Worthington). To 100- μl aliquots of HLA-B7 solution (at 125 μg ml^{-1}), in 100 mM Tris-HCl pH 8.0, 25 mM EDTA, 10 mM CaCl_2 , 2.5 mM dithiothreitol and 10 mM putrescine, 10 μl of papain were added and the solution was incubated for 1 h at 37 °C. The reaction was stopped by transfer to ice and by addition of 10 mM iodoacetic acid. The samples were trichloroacetic acid-precipitated and prepared for electrophoresis as in Fig. 1 legend. In this experiment an 8-cm 12.5% acrylamide gel was used with the buffer system of Laemmli¹¹. Lanes 1–4 represent proteolysis by 0, 0.2, 1 and 5 μg of papain, respectively. Lane 5 is a rabbit skeletal muscle actin standard. *a* Shows the gel stained with Coomassie blue and *b* is a fluorograph of the same gel. Note that ³H-putrescine label was excised concomitantly with release of the intracellular, hydrophilic portion of the HLA-B7 heavy chain as p44 was converted to p39.

receptor-mediated uptake⁷ and response to mitogenic lectins⁸, by catalysis of the cross-linking of the intracellular portion of a transmembrane protein to other intracellular constituents. Labelling of HLA-A and -B antigens (or any other membrane proteins) in intact or broken peripheral blood lymphocytes or lymphoblastoid cells incubated with ³H-putrescine or dansyl cadaverine has not been detected (J. P. and B. Guild, unpublished observations), but suitable conditions may not have been used. However, the data presented here establish that one

common membrane protein in its intracellular domain is a suitable substrate for transglutaminase-catalysed reactions.

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(1→3)- β -D-Glucan (callose) is a probable intermediate in biosynthesis of cellulose of cotton fibres

H. Meier, L. Buchs, A. J. Buchala & T. Homewood

Institut de Biologie végétale et de Phytochimie,
Université de Fribourg, CH 1700 Fribourg, Switzerland

The biosynthetic pathway of cellulose in higher plants is unknown¹. Attempts to produce cellulose *in vitro* have shown that mainly (1→3)- β -D-glucan (callose) is produced from particulate enzyme fractions of higher plants and uridine diphosphate glucose (UDPG)²⁻⁵. Some authors have claimed that in certain conditions, (1→4)- β -D-glucosidic linkages were also formed⁶⁻¹⁰. However, there is no conclusive evidence for the formation of pure (1→4)- β -D-glucans from UDPG. As callose forms naturally not only in various parts of plants (sieve and pollen tubes, root hairs, cell plates) but also in response to wounding, it seems best to study cellulose biosynthesis *in vivo* rather than in wounded cells or cell homogenates which might produce callose but not cellulose. Using intact cotton fibres, we have now shown by pulse-chase experiments that callose has a high turnover and is likely to be an intermediate in cellulose biosynthesis at the stage of secondary wall formation.

Naturally grown cotton fibres contain large quantities of callose¹¹⁻¹³. In the fibres of one ovule of *Gossypium arboreum*, at the onset of secondary wall formation (about 25 days after anthesis), ~1 mg of callose is present out of a total fibre wall weight of ~13 mg. Using two experimental systems, we have investigated the incorporation of ¹⁴C-labelled compounds into the cell wall polysaccharides of intact seed hairs of cotton (*Gossypium arboreum* L.). In the first system, whole fruit capsules received, through the cut surface of the petiole, a pulse of [¹⁴C]sucrose (for about 20 min) followed by a chase with unlabelled sucrose for periods of up to 40 h (Fig. 1 legend). Six

capsules, collected about 35 days after anthesis (at the stage of secondary wall formation), were treated in this way. At various intervals they were opened quickly and the intact seed clusters were immediately immersed in boiling 80% (v/v) methanol to avoid possible formation of wound callose. The fibres were isolated, further extracted with hot 80% methanol to remove low-molecular weight substances, and the methanol extracts combined; the fibres were finally homogenized. The radioactivity in the 80% methanol-soluble fraction was mainly contained in sucrose and may be taken to indicate the size of the low-molecular weight precursor pool at the time of analysis. The radioactivity in aliquots of the fibres was counted and other aliquots were treated with dimethyl sulphoxide (DMSO) to extract non-cellulosic high-molecular weight material¹⁴. The radioactivity in the latter and in the residues was measured. When the DMSO-soluble high-molecular weight material was hydrolysed, glucose was the chief product and the only labelled sugar obtained, although small amounts of unlabelled galactose,

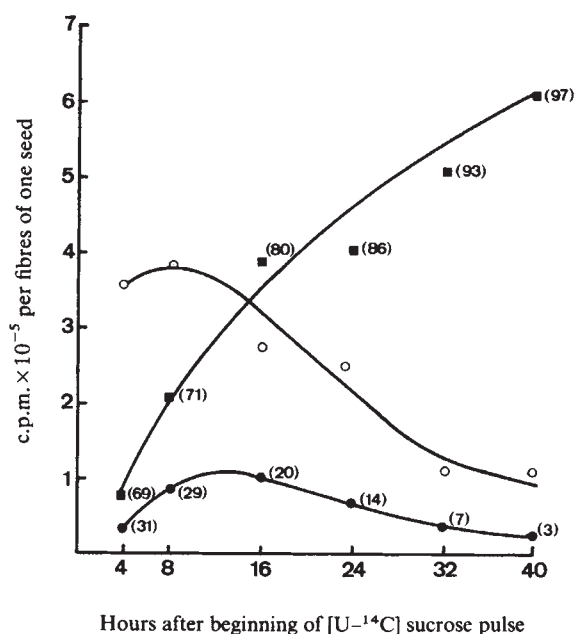


Fig. 1 Pulse-chase feeding of sucrose to petioles of fruit capsules of *Gossypium arboreum* L. The capsules were harvested 35 days post-anthesis (stage of secondary wall formation). The pulse of [¹⁴C]sucrose (20 μ l, 1 mM, 1.85 MBq) was applied through the cut ends of the petioles for about 20 min. The chase was with unlabelled sucrose (~50 μ l h⁻¹, 1 mM) in the dark at 30 °C. After different incubation periods the radioactivity was measured in the 80% methanol-soluble fraction (○), the DMSO-soluble fraction, that is, (1→3)- β -D-glucan (callose) (●), and the residue, (1→4)- β -D-glucan (cellulose) (■), of the fibres. Numbers in parentheses are the percent radioactivity in callose- and cellulose-type glucans, respectively.

arabinose and xylose were detected. Methylation studies and radio gas chromatography, as well as degradation with exo-(1→3)- β -glucanase (EC 3.2.1.58) from *Basidiomycete* sp. QM 806, showed that the labelled glucan in the DMSO extract was of the (1→3)- β -type (callose-type) with a few (1→6)-linkages (our unpublished results). Further treatment of the residual fibre homogenate after DMSO extraction with the exo-(1→3)- β -glucanase gave only very minor amounts of glucose, indicating that with DMSO essentially all the (1→3)- β -D-glucan had been

removed. No degradation was detected on treatment with lichenase (from *Bacillus subtilis*), α - or β -amylase, thereby excluding the possibility of the presence of other non-cellulosic glucans known to be present in higher plants. Acetolysis of the residue gave the peracetate derivatives of glucose, cellobiose and higher cellobiosaccharides. The cellobiose octaacetate was obtained in about 20% yield and such a value is usually taken to indicate that the polysaccharide in question is cellulose. This product was characterized by melting point, mixed melting point and TLC, and its specific activity, which was the same as that of the fibre residue, remained constant on recrystallization. These results show that the radioactivity of the fibre residue is essentially confined to cellulose.

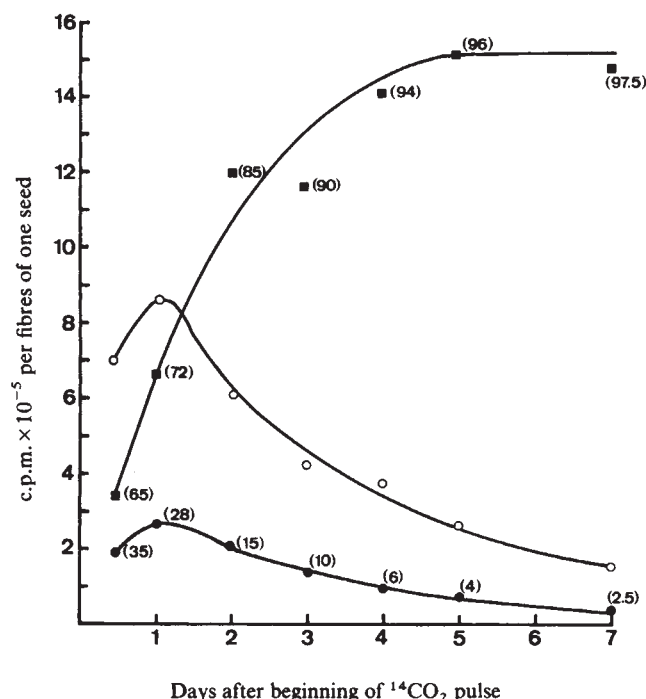


Fig. 2 Pulse-chase feeding of $^{14}\text{CO}_2$ to branches of intact plants of *Gossypium arboreum* L. $^{14}\text{CO}_2$ (7.4 MBq) was fed in the morning in bright sunlight to branches, each with three or four leaves and one capsule at 35–40 days post-anthesis, inside polythene bags. After 30 min about 80% of the original radioactivity in the bags had been taken up by the branches which were, however, left inside the bags for another 90 min. The latter were then removed and the plants were left in normal day and night conditions, when photosynthesis could occur normally, until the capsules were harvested. The radioactivity was determined in the 80% methanol-soluble fractions (○), the (1 $\rightarrow$ 3)- β -D-glucan (callose) (●) and the (1 $\rightarrow$ 4)- β -D-glucan (cellulose) (■) fractions of the fibres. Numbers in parenthesis are the percent radioactivity in the callose- and cellulose-type glucans, respectively.

Figure 1 shows that 4 h after the beginning of the pulse a high proportion (31%) of the radioactivity in cell wall glucans is confined to callose. The total radioactivity in the callose increased for about 16 h and then decreased continuously whereas the radioactivity in the cellulose continued to increase. A turnover of callose is evident; its radioactive content decreased from 100,000 c.p.m. in the fibres of one seed to 20,000 c.p.m. between 16 and 40 h after the pulse, while in the same period the radioactivity in the cellulose increased from about 400,000 to 600,000 c.p.m. This larger increase in the radioactive content of the cellulose is readily explained by the

fact that 16 h after the pulse, there were still 280,000 c.p.m. in the fibres of one seed confined to low-molecular weight precursors (methanol extract). The apparent turnover of callose is relatively slow but this may be due to the slow replacement of radioactive sucrose by the unlabelled sucrose during the chase, or to the time delay in the arrival of sucrose to different fibres on different seeds in the capsule.

In the second system, $^{14}\text{CO}_2$ was given for about 2 h to branches of intact cotton plants with one capsule per branch. This seems to be the most natural experimental system in which abnormal wound reactions would be excluded. In this system also (Fig. 2), within a relatively short time after the pulse a high proportion (35%) of the 80% methanol-insoluble radioactivity of the fibres was in callose. The absolute radioactive content of the callose increased until about 1 day after the pulse and then decreased, whereas that of the cellulose continued to increase. The radioactivity in callose relative to that in cellulose decreased throughout the experiment. The radioactivity in the 80% methanol-soluble low-molecular weight substances of the fibres reached its maximum about 1 day after the beginning of the pulse and decreased thereafter. These results also indicate a turnover of callose and the probable incorporation of its glucose residues into cellulose.

A sharp chase with the nonradioactive compound was impossible with either of the two systems because of the time lag necessary for the entry of the chase substance into the fibre pool and because a certain amount of radioactive precursor continued to enter the fibre precursor pool from other parts of the fruit capsule. It is therefore feasible that the apparent rate of decrease in the radioactivity of the callose during the chase is lower than the apparent rate of increase in the radioactivity of the cellulose even if callose is an obligatory precursor for cellulose. Although our results, in contrast to those of Maltby *et al.*¹⁵, indicate a clear turnover of callose, they do not show whether or not callose is a direct glucosyl donor for cellulose. If this is the case, a (1 $\rightarrow$ 3)- β -D-glucan:(1 $\rightarrow$ 4)- β -D-glucan glucosyl transferase (transglucosylase) would probably be involved in cellulose synthesis. (1 $\rightarrow$ 3)- β -D-Glucanases are undoubtedly present in cotton fibres (unpublished results) and we are now investigating whether, in certain conditions, they can act as transglucosylases.

Suggestions that callose is a cellulose precursor are not new and have been made by several plant cytologists^{16–18}. Waterkeyn (personal communication) showed by fluorescence microscopy, after staining with aniline blue, that at different stages of secondary wall formation, cotton fibres always contain a thin layer of a substance reacting like callose at the inner side of the wall. If further studies confirm the precursor role of callose for cellulose in cotton fibres as well as in other cell types, it could be argued that callose may accumulate where the biosynthetic step from callose to cellulose is inhibited.

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MATTERS ARISING

Allez Neanderthal

APSIMON¹ concluded that Lévêque and Vandermeersch's discovery of a Neanderthal specimen at Saint-Césaire associated with Châtelperronian denies a Neanderthal ancestry for modern Europeans. In reality, taking account of other evidence a completely different conclusion may be drawn from this critical discovery.

The following points are relevant. (1) Robust but modern (although not European) populations are associated with Mousterian (Middle Palaeolithic) industries in the Levant^{2,3}. (2) Neanderthal (or Neanderthal-like) remains are associated with Aurignacian (Upper Palaeolithic) in Croatia⁴⁻⁶. (3) Evolutionary trends in the European Neanderthals themselves (*contra*⁷) are clearly in the direction of modern Europeans^{6,8}. (4) The earliest modern Europeans (earlier than Cro-Magnon) are distinctly Neanderthal-like in their morphology^{5,8-10}.

The evolutionary hypothesis is supported by the backward extension of trends in the modern Europeans (the samples become distinctly more Neanderthal-like earlier in time) and the forward extension of trends in the Neanderthals of Europe (they become distinctly more like modern Europeans later in time). In this regard, Saint-Césaire specifically resembles the late (more modern) Neanderthals from Vindija cave⁴⁻⁶ and thus provides important support for the observed trends within the Neanderthal sample.

The average changes between late Neanderthals and early modern Europeans should not be confused with the comparison of La Chapelle and the Cro-Magnon male. Such changes could happen fairly quickly, responding to changes in skeletal stress during growth¹¹ and changes in selection⁵. If migration played an important role, the origin of the migrating populations with their distinctive European features remains unknown. If the Levant is considered to be the place of origin, in spite of the lack of morphological relations, the resulting juxtapositions of hominids and industries makes nonsense of virtually every previous hypothesis of archaeological or morphological sequence.

MILFORD H. WOLPOFF

Department of Anthropology,
University of Michigan,
Ann Arbor, Michigan 48109, USA

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APSIMON REPLIES TO WOLPOFF—We are in agreement about point (1). Concerning point (2): The relevant hominid finds (from Vindija, layer G₁) are described as 'non-diagnostic morphologically' and could be Neanderthal or early *sapiens*, the lithic artefacts from the layer are not diagnostic, but the single split-based bone point is consistent with 'Aurignacian'. There is, however, no intrinsic improbability about Neanderthal—Aurignacian association. I agree with points (3) and (4). Analyses which bring together in one sample all classic European Neanderthal crania and in another sample all European Upper Palaeolithic crania (including some specimens now considered to be Mesolithic) obscure this. The recently described Neanderthal frontal bone from Hahnöfersand near Hamburg¹, dated to ~36,000 BP, which has been considered as indicating hybridization with *sapiens*, might equally well be cited as a further example of this evolutionary trend.

In general, the hypotheses presented by Wolpoff provide an explanation of the Neanderthal—*sapiens* transition in Europe which can be far more easily harmonized with current interpretation of the archaeological evidence than can the hypotheses presented by Stringer *et al.* However, if the archaeologist must pick his way with care through the differing interpretations offered by anthropologists, the latter would be well advised to remember that 'Upper' and 'Middle Palaeolithic', 'Mousterian', 'Aurignacian' and such are artificial constructions, that attempts to attribute artefactual assemblages to one or other category may create impressions of discontinuity where none exists, and that the most useful information to be obtained from artefacts relates to patterns of activity.

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WE are in broad agreement with APSIMON¹ regarding the significance of the Saint-Césaire discovery from France², although it must be emphasized that our comments are preliminary in the absence of detailed descriptions of the archaeology and fossil human material. An effect of

this discovery will be to focus the 'Neanderthal problem' in Europe to a consideration of events in the period between ~35,000 and 30,000 yr b.p.

The discovery of early Upper Palaeolithic tools in association with a Neanderthal skeleton at Saint-Césaire has apparently confirmed the prediction that the technological transition to the Châtelperronian was accomplished by indigenous populations³. It is now plausible that the same kind of association between Neanderthal hominids and industries termed 'early Upper Palaeolithic' applies for other areas in Europe (for example, for the 'leaf-point' industries of northern and central Europe and for the Uluzzian of Italy)⁴. Furthermore, the cultural continuity of these industries (for example, the Châtelperronian) with later Upper Palaeolithic industries (the later Perigordian [Gravettian] for example) has by no means been established because consideration of the subsequent development of these early Upper Palaeolithic industries often reveals the intervening presence of Aurignacian assemblages which are typologically not locally derived. In France there are occurrences of both Châtelperronian and Aurignacian, but during the subsequent Arcy interstadial (~32,000–31,000 yr b.p.) the Châtelperronian disappears^{5,6}.

ApSimon's claim that there is no reliable evidence for the existence of the Aurignacian complex in central Europe earlier than it occurs in France needs qualification. Although the Tŕnovo province of Bulgaria is not geographically central in Europe, recent work there at the Bacho Kiro cave is highly germane to a discussion concerning the origin of the Aurignacian in Europe. For this site there is now an internally consistent series of radiocarbon dates which supports the existence of a well defined Aurignacian industry at about 43,000 yr b.p. (Ly-1102, GrN-7569, 7545, 7570) which has no local antecedents. It is claimed that the same stratum is associated with 'primitive' but anatomically modern human remains^{7,8}. If we accept these dates from Bacho Kiro it raises the possibility that the Aurignacian there is ancestral to that of central and western Europe. However a postulated east-west diffusion of Aurignacian populations and/or techniques cannot yet be demonstrated with the present precision of the radiocarbon method.

In south-west Asia the critical time period available for an evolutionary transformation or replacement of Neanderthal populations is probably before 40,000 yr b.p., perhaps well before⁹. Thus the Saint-Césaire find apparently demonstrates for the first time that

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2. McCown, T. D. & Keith, A. *The Stone Age of Mount Carmel* 2 (Clarendon, Oxford, 1939).

Neanderthals in western Europe probably existed at the same date as much more modern-looking populations in south-west Asia (as at Skhul and Qafzeh) and perhaps in Europe (as at Velika Pećina, Brno, Mladeč (*contra* ApSimon) and Hahnöfersand)¹⁰⁻¹⁶. No well preserved fossils which are demonstrably intermediate in significant characters between Neanderthals and anatomically modern humans have been described from either of these areas. The fact that some of the earliest anatomically modern hominids in these areas display characters such as robust brow-bridges and large teeth does not necessarily imply that they evolved from Neanderthal ancestors, as such characters are probably merely plesiomorphous (primitive) for *Homo sapiens* generally, and therefore have no phylogenetic relevance in these comparisons. As European and south-west Asian Neanderthals share suites of characters which are apparently autapomorphous (uniquely derived), it is these traits which are significant in any analysis of relationship between Neanderthal and early anatomically modern hominids, as is the presence or absence of traits considered autapomorphous for anatomically modern hominids. With very few exceptions such characters do not cross the morphological division between the Neanderthal and early anatomically modern groups^{10-13,17}.

The many characters shared between the European and south-west Asian Neanderthals and the many different characters shared between the early anatomically modern fossils of the same two areas mitigates against any evolutionary scheme which posits only *in situ* evolution between Neanderthals and anatomically modern humans in each area^{10-13,17}. Evidence now suggests that Eurasian Neanderthals were temperate or cold-adapted forms, whereas the early anatomically modern humans of the same area were not. This implies that a model involving at least some gene flow from outside this area at the time of the first appearance of anatomically modern humans is more appropriate¹⁸.

Thus we agree with ApSimon that, pending further description, the Saint-Césaire Neanderthal disproves the idea of an *in situ* hominid transformation in Europe at a Mousterian/Upper Palaeolithic interface. Instead, the polyphyletic origins of the Upper Palaeolithic in Europe may be matched by a dichotomy, at least, in the morphology of the hominids that produced it. The wealth of new evidence that is now accumulating, together with the promise of a more reliable chronological framework, will allow us to monitor more precisely the events of this key period in prehistory. We would also argue that more sophisticated models are needed than those which posit either mass extinction, hybridization or rapid *in situ* evolution of Neanderthal hominids at

the time of the appearance of anatomically modern humans in Europe.

C. B. STRINGER

R. G. KRUSZYNSKI

Department of Palaeontology,
British Museum (Natural History),
Cromwell Road, London SW7 5BD, UK

R. M. JACOBI

Department of Classics and Archaeology,
Lonsdale College,
University of Lancaster, Bailrigg,
Lancaster LA1 4YN, UK

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APSIMON REPLIES TO STRINGER ET AL.—Concerning Bacho Kiro: If the industry corresponds to accepted definitions of 'Aurignacian', 43,000 BP would be a surprising date, as it would be ~10,000 yr older than dates obtained elsewhere.

We can agree that Velika Pećina, Brno and Mladeč may be of the same order of age as Saint-Césaire, subject to the uncertainties of present chronologies, but not that they might be substantially older, as was implied by the traditional interpretation of Würm I-II-III in central Europe.

I do not believe that the case has yet been made for the previous evolution of the Aurignacian. Interstratification of Aurignacian and Châtelperronian raises the possibility that they are functionally differentiated variants of the same tradition. Blade technology is not substantially more important in these early European 'Upper Palaeolithic' industries than in some Mousterian industries and development from the Mousterian lithic tradition seems to be their most likely origin.

ARTHUR APSIMON

Department of Archaeology,
University of Southampton,
Southampton SO9 5NH, UK

Why do jackals help their parents?

BLACK-BACKED jackals (*Canis mesomelas*) sometimes stay with their parents and help them raise subsequent litters. Based on a positive correlation between the number of pups surviving in a litter and the number of adults (parents plus helpers) in the family unit, Moehlman¹ concluded that pup survivorship is enhanced by the presence of helpers. Unfortunately, her analysis contains errors of calculation and interpretation that seriously weaken this conclusion. Here I correct those errors and present an alternative explanation for her results.

First, an error in the calculation of the correlation coefficient (between 'pups surviving' and 'number of helpers') markedly inflates the statistical significance of the result. From the data shown in Moehlman's Fig. 1, I calculate $R_s = 0.58$ (Spearman rank correlation coefficient; $P < 0.05$) and not $R_s = 0.967$ ($P < 0.01$) as reported. Although the correlation coefficient is still statistically significant, factors other than the number of jackal helpers in a family unit obviously contribute substantially to the observed variation in the number of pups that are raised from a litter.

Second, it is stated¹ that "an adult helper gains more (yield 1 pup per adult) by being a helper of its parents than by finding a mate and raising its own pups aided only by its mate (yield $\frac{1}{2}$ pup per adult)." For both parents and helpers, however, the relevant measure of reproductive success is the total number of pups that survive from a litter, and not the number that survive per adult. As long as a helper's parents stay mated, the coefficients of relatedness between the helper and its siblings in subsequent litters, and between the parents and their new offspring, are the same (that is, $r = \frac{1}{2}$). Therefore, a helper realizes the same 'reproductive success' whether helping its parents increase their litter size by n pups or raising n pups itself (with its own mate). The calculation of 'yield per adult' always underestimates the benefit that accrues to helpers when they contribute to the increased reproductive success of their parents. For example, in terms of yield per adult, a jackal seems to increase its reproductive success by only $n/3$ when helping its parents raise an additional n pups, but it increases its reproductive success by $n/2$ by raising its own n pups. In fact, both of these increases in reproductive success should be the same.

Data shown in Moehlman's Fig. 1 can now be used to assess the reproductive consequences of becoming a helper instead of mating. The slope of the geometric mean regression² of 'pups surviving' over 'number of helpers' ($y = 0.9 + 1.67x$; $n = 15$) is an estimate of the

average increase in reproductive success of a family unit due to the addition of each helper. Thus, each helper adds about 1.7 pups to a litter raised by its family unit. The reproductive success of an adult that finds a mate and raises pups, without any helpers, is, on average, 1.0 pups ($n = 4$). In the short term, then, jackals do seem to gain more (a net gain of $1.7 - 1.0 = 0.7$ pups) by helping their parents than by mating, but these gains must ultimately be weighed against the disadvantages of foregoing both dispersal and mate selection until the family unit breaks up (for example, when one of the parents dies). It

(*Todus mexicanus*)⁵, where clutch sizes are larger when families have helpers. Jackals may ultimately help their parents because helping increases their inclusive fitness but further work will be needed to determine whether those fitness increases are due to larger litter sizes or increased pup survival.

ROBERT D. MONTGOMERIE

Department of Biology,
Queen's University,
Kingston, Ontario, Canada K7L 3N6

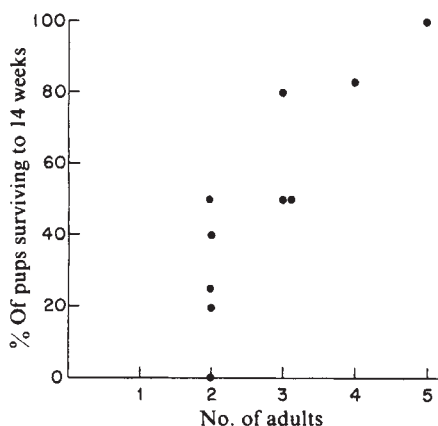


Fig. 1 Number of adults per family and pup survivorship from 3 to 14 weeks ($n = 10$; Spearman rank correlation coefficient, $R_s = 0.88$; $P < 0.01$).

is not surprising, therefore, that few jackals remain with their parents for more than 1 yr (ref. 1).

Finally, Moehlman erroneously equates 'number of pups surviving' with 'pup survivorship'. Survivorship means survival rate, and would be an estimate of the average probability of a pup surviving to weaning, but such data are not presented. The litter size of black-backed jackals at parturition varies from one to eight pups³. I suggest, therefore, that the positive correlation between the number of helpers and the number of pups raised may not be caused by pup survival rates, but may be the result of higher litter sizes at birth for females that have helpers. Helpers are usually young jackals that have stayed with the family unit from a previous litter¹. Their continued presence could, therefore, act as a proximate stimulus for their mother to increase litter size, especially as helpers also provide food for the family unit¹. This pattern should be looked for in other studies of communal breeding systems and may be expected in any species where clutch size varies with food supply. For example, such a response to helpers seems to occur in both the Tasmanian native hen (*Tribonyx mortierii*)⁴ and the Puerto Rican Tody

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MOEHLMAN REPLIES—Montgomerie is correct in recalculating $R_s = 0.58$ (Spearman rank correlation coefficient; $P < 0.05$). I thank him for his correction; however, I disagree with his alternative explanation, in that he implies it is mutually exclusive.

The correlation coefficient is still significant. Furthermore, litter no. 15 was an aberrant group in which the parents were old¹, only one helper of three was functional throughout the critical period of pup development and one pup survived. This particular helper was also the only example of an individual helping for more than 1 yr. This datum could be shifted to the three-adult column, but for biological reasons I think that it distorts the relationship between presence of helpers and pup survival. If correlation coefficients are calculated for the remaining 14 litters, $R_s = 0.83$ ($P < 0.01$).

The relevant measure of reproductive success is the number of genes surviving. Thus Montgomerie is correct in stating that the important measure is the total number of surviving, related pups. Additional data for three litters were collected in 1978, and on average, in 17 litters: one pair raised 1.3 pups ($n = 6$); one pair + 1 helper raised 3.3 pups ($n = 8$); one pair + 2 helpers raised 4.0 pups ($n = 2$); one pair + 3 helpers raised 6.0 pups ($n = 1$). (The data on litter 15 are not included in these calculations.) Thus, by delaying reproduction for 1 yr, a helper potentially increases its inclusive fitness by on average 1.7 pups, assuming that helping incurred a minimal cost. At present there is controversy as to how to measure 'inclusive fitness' and any suggestions would be appreciated. This, of course, is only one variable, and factors such as increased experience on the natal territory, territorial inheritance and increased survivorship may be equally important (from the helper's point of view). The situation is further complicated by the fact

that a 1-year-old jackal can emigrate and still accrue this inclusive fitness as long as a sibling stays and helps. Data on success at emigrating, mating and establishing a territory at 1 yr of age compared with 2 yr of age are needed to answer fully questions concerning kin selection in this study population of black-backed jackals.

Number of adults versus number of live pups at 14 weeks is the critical measure in terms of kin selection and investigating the problem of why jackals help their parents. However, to clarify the relationship between the presence of helpers and pup survival, further data are presented (Fig. 1). Litter size at first emergence from the den (approximately 3 weeks old) is known for 10 of 17 litters. Dens were not disturbed because of the potential detrimental effect on pup survival and subsequent den use (dens may be used again). The mother spends most (90%) of her time in the den during the first 3 weeks, and is fed by her mate and helpers. Data presented in ref. 2 was for pup survival to 14 weeks. As stated, it was during this vulnerable period that mortality was observed. Thereafter, pups no longer used a den and began to forage throughout the home territory. Number of adults versus pup survivorship (3-14 weeks): $n = 10$, $R_s = 0.88$, $P < 0.01$; number of adults versus pup survival at 14 weeks: $n = 17$, $R_s = 0.85$, $P < 0.01$; number of adults versus litter size at 3 weeks: $n = 10$, $R_s = 0.83$, $P < 0.01$; number of pups at 3 versus 14 weeks: $n = 10$, $R_s = 0.68$, $P < 0.05$.

The highest R_s value is between number of adults and percentage of pups surviving from 3 to 14 weeks. The lowest R_s value is between litter size at 3 weeks and 14 weeks. Information on gestation and prenatal growth in mammals suggests that pup size rather than number of pups will be affected by maternal nutrition, except in cases of prolonged starvation³. Litters of dogs raised in the laboratory experience high mortality during the first 3 weeks¹. Thus information on litter size at 3 weeks may not be a reliable indicator of initial litter size. Helpers may increase initial litter size, but in current data obtained with jackals, the most significant correlation is with pup survivorship from 3 to 14 weeks. Thus it seems reasonable to conclude that helpers improve pup survival rates, possibly from a very early age, and may even affect initial litter size.

PATRICIA D. MOEHLMAN

Department of Zoology,
University of Wisconsin,
Madison, Wisconsin 53706, USA

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Size constancy does not fail below half a degree

AS an observer's distance from an object varies, so does the visual angle subtended by the object at the observer's eye. One of the most reliable phenomena in psychology is that when distance information is available the perceived size of an object is nearly constant despite variations in visual angle, or retinal image size¹. A corollary is that images of constant visual angle seem to vary in size with perceived distance (Emmert's law). This phenomenon ('size constancy') is only one part of the more general property of perception that one might call 'object constancy', embracing constancy of size, shape, depth, velocity, lightness, colour and so on. A recent report² has claimed "that size constancy holds when image size is above half a degree, and breaks down when it is less". The claim, however, was based on demonstrations and anecdotal evidence, rather than experiment. We show experimentally that size constancy in the laboratory is equally good at target sizes above and below $\frac{1}{2}^\circ$.

The targets were single, black, vertical lines 1.2 arc min wide presented at three viewing distances (114, 228 and 456 cm) with four lengths ($\frac{1}{8}, \frac{1}{4}, \frac{1}{2}, 1$ deg). Each line was fixed in the centre of a white card subtending 5.25° vertically by 3.75° horizontally independent of viewing distance. Each card was placed at the appropriate position on a long laboratory bench on which papers and small objects were scattered. Viewing was binocular with full room lighting. Thus, distance information was abundant, but for a given target size (angle), the length and width of the line and of the frame in which it was set were of constant visual angle, independent of distance. The subject looked back and forth between the target and an oscilloscope screen placed at 228 cm distance, about 6° to the right of the target. His task was to adjust the vertical separation between two small green dots on the screen until the separation 'appeared to match the size of the target'. Trials were presented in random order and the 12 conditions were repeated 3 times for each of the 10 subjects (5 male, 5 female).

The results (Fig. 1) show clearly that perceived length of a line of constant visual angle varies with distance in almost exactly the same way for all target sizes in the range $\frac{1}{8}^\circ$ – 1° . Analysis of variance not surprisingly showed highly significant effects of both visual angle and viewing distance ($P < 0.001$), but most importantly the interaction between distance and visual angle was not significant ($F_{6,29} = 1.47$, $P > 0.10$). If size constancy were weaker or absent below half a degree the slope of the curves in Fig. 1 would be shallower or even flat for small target

lengths. Our data strongly contradict any such effect. Constancy was not complete (this would be given by a slope of 1) but it was equally good for all target sizes.

In a second part of the experiment, an electronic flashgun was used to create the afterimage of a line $\frac{1}{4}^\circ$ long. The observer viewed the afterimage on a plain white card of constant visual angle at each of the three distances, and made length matches as in the first part of the experiment. A second or third exposure of the line was given if necessary to complete a total of nine trials. One subject reported no afterimage, but the data from the remaining nine (crosses in Fig. 1) were not significantly different from those obtained with a real line of $\frac{1}{4}^\circ$ ($F_{1,8} = 0.84$, not significant).

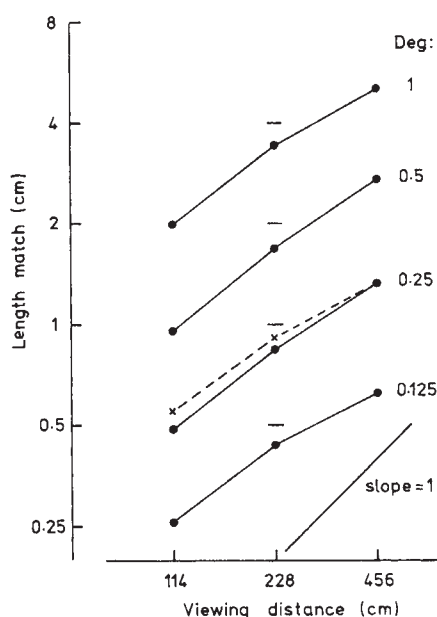


Fig. 1 Solid symbols show results averaged over 10 subjects who adjusted a pair of dots to match the lengths of lines subtending four different visual angles at three viewing distances. Crosses represent similar judgements (nine subjects) made with an afterimage $\frac{1}{4}^\circ$ long. Horizontal bars represent the ideal match when target and dots were at the same distance. A slope of 1 would represent perfect constancy, and zero slope would indicate judgements based purely on visual angle (no constancy). The average slope of regression lines fitted to the solid points was 0.70, with no significant interaction between distance and visual angle.

Ross *et al.*² reported that afterimages subtending less than $\frac{1}{2}^\circ$ did not seem to change size with 'projected' distance, and that the finding was robust in classroom demonstrations. We flashed a 2-cm line to a group of 27 students, all of whom were

more than 228 cm distant. The afterimage would thus be less than $\frac{1}{2}^\circ$ for all observers. They observed the image on their hands and then on a far wall. None reported no change of size, 19 said the image got bigger when further away, 2 said it got smaller and 6 reported no image. Ross *et al.*² also appeal to the reader's direct experience of the breakdown of constancy. Our own experience confirms our classroom demonstration, that small afterimages obey Emmert's law in the usual way. When we acted as subjects in the experiment, our data were virtually identical to those of Fig. 1.

Ross *et al.*² remark that objects seen in the distance or from the top of a tall building seem 'small'. It may be that in these cases, and in their Fig. 1, information about the true distance is inadequate, or impoverished by poor resolution of detail. As a result, both distance and size would be underestimated¹. The fact that many distant objects subtend less than $\frac{1}{2}^\circ$ may be quite incidental.

We conclude from our experiments and demonstrations that size constancy for both real targets and afterimages is just as good for small images as for large ones. Indeed, we should suffer very curious distortions in the perceived relative size of objects if this were not so. There seems to be no substance to the claim that size constancy fails below half a degree.

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M. A. GEORGESON
M. G. HARRIS

University of Bristol,
Department of Psychology,
8-10 Berkeley Square,
Bristol BS8 1HH, UK

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Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

BOOK REVIEWS

Risk assessed and risk perceived

Eric Ashby

Societal Risk Assessment: How Safe is Safe Enough? Edited by R.C. Schwing and W.A. Albers Jr. Pp.363. (Plenum: 1980.) \$29.50, £18.59.

LOOKED at with the naïve eye of logic, a calamity killing 10^4 people, likely to occur once every 10^4 years, does not appear different from a calamity killing one person every year. Looked at with the complex eye of human judgement, there is a massive difference. A nuclear melt-down once every ten years, killing half a million people, is a horrifying thought; but that level of carnage is only the linear extrapolation of the average annual death rate on the roads of the United States. This disparity between the statistical assessment of a risk and the personal perception of the risk has important implications for public policy. The cost of making things safe — roads, airlines, nuclear power stations, drugs — rises steeply as the risks are reduced. A level of cost is reached when it would be fantastically expensive to make the thing any safer. So politicians have to ask: How safe is safe enough?

It is not surprising, therefore, that conferences and books on risk-management are cropping up all over the place. They bring together the opinions of statisticians, who can tell you with some confidence what your chances are of being killed on the roads, stifled with emphysema through smoking cigarettes, or struck by lightning; and the opinions of psychologists, who can tell you (with as much confidence, if with less evidence) how you and I evaluate these statistical assessments. And the conferences are attended by policy-makers who go away perplexed and under the obligation to make decisions which will reconcile the disparity between assessed and perceived risks. The disparity remains, but it is encouraging that conferences on risk-management bring to the surface new ideas and fresh attitudes to a problem which has become of major political importance.

Societal Risk Assessment is a record of one of the best recent conferences on the issue. It was arranged by the General Motors Research Laboratories. Let it be said at once that although General Motors might be assumed to have a vested interest in reassuring the public about some kinds of risks, there is no sign of bias in this volume. There are frank discussions of the risks we run and the risks we accept; this section includes an excellent summary of the causes of road accidents, studied in England by a team at the Transport and Road Research Laboratory. The book then

goes on to discuss what it costs to reduce risks. Notable among these discussions is a paper from an expert on underwriting in the insurance business. When you come to think of it, insurance underwriters are the one profession who have to try to reconcile assessed and perceived risks; otherwise they would go out of business.

Having discussed what are, and what are not, acceptable risks, and how much society can afford to pay to reduce risks, the conference then went on to tackle an even more controversial question: Who shall decide what risks we are permitted to run? This is a touchy issue among motorists in Britain just now. Despite the alarming casualty figures on the roads of Britain (nearly 7,000 a year killed) the chance of any individual in the whole population being killed is only 1 in 2,500 years. This explains the indifference of the British public toward road accidents. But the problem for society as a whole remains serious: every fatal accident is reckoned to cost some £64,000. It is not disputed that the number of fatal and serious accidents would be reduced if people wore seat belts, drove at not more than 55 miles an hour (as they are obliged to do in many American States), and did not drink alcohol before driving. Is it a duty of the State to compel its citizens to take these precautions? And if so, can the citizens be persuaded by propaganda to come to a consensus about this (after all, there is a consensus about driving on the left-hand side of the road)? Or must they be compelled without having consented? Or, in the name of liberty, should motorists be allowed to endanger themselves (at the taxpayer's expense in a country with a national health service) to their heart's content? The conference did not, of course, come to decisions on these

questions; but the issues were thoroughly aired.

The first three sections of the book endorse, with a wealth of fresh examples and neat arguments, conclusions already familiar to those who have studied the subject: for example that there is no such thing as zero risk; that people (including experts) commonly bias their opinions by giving too much weight to the severity of an incident and too little weight to its frequency; that unfamiliar risks, such as hazards from radioactivity and chlorofluorocarbons, generate more anxiety than "classical" risks, such as floods and tornadoes. The last section of the book tackles the consequence of these conclusions, namely that "at the center of the risk problem are people and their fears". Life in affluent Western countries is more secure than it has ever been; yet people are more jittery about hazards than their grandparents ever were. There are still highly educated people who will not sit down to dinner with 13 at the table, and other highly educated people who join expeditions to climb in the Himalayas. The last three papers in the book deal with this fascinating region of social anthropology.

There is already a considerable library of books on risk assessment. Two of the books are outstanding in quality and content. One is the report on the international seminar held in Stockholm in 1978 (*Energy Risk Management*, edited by G.T. Goodman and W.D. Rowe; Academic Press, 1979). The other is *Societal Risk Assessment*. Anyone who has digested these two books can reckon to be up to date in the state of the art of risk management. □

Lord Ashby is Chancellor of Queen's University, Belfast, and a Fellow of Clare College, Cambridge.

Electron microscope? Better buy the book!

R. K. S. Wood

Fungus Diseases of Tropical Crops. By Paul Holliday. Pp.607. (Cambridge University Press: 1980.) £55, \$125.

A LARGE proportion of the world's crops are grown in the tropics where they are of two main kinds. First are those with cash products, important in world trade and grown in large units using advanced technologies. Then there are the crops, not always of food, grown in much smaller units by methods still largely traditional

and little affected by modern technology. As would be expected, there is substantial research on diseases of the first type of crop, but much less on diseases of the other, although, of course, these diseases are as or more important when they decrease the yields of subsistence foods to tragically low levels. Diseases of food crops in temperate countries may be financially inconvenient; in the tropics they can cause famine.

There are many general texts and

innumerable monographs on diseases of temperate crops — understandably, because most of the world's plant pathologists are in non-tropical countries. In relation to need there are all too few authoritative texts on diseases of tropical crops; the publication of a new and large book on the subject is, therefore, something of an event.

Dr Paul Holliday has been studying and reporting on diseases of tropical crops for about 30 years, many of them as a member of a pool of plant pathologists, spending much of his time in the tropics and the rest of it at the Commonwealth Mycological Institute at Kew. With this background, there was every reason to expect a first-class book from the author, and first class it is.

An introduction describes in detail the plan of the book and its parts, and the main sources of information on taxonomy and nomenclature. There follow, in 556 double-column pages of small but readable type, descriptions of fungal pathogens and the diseases they cause in plants grown as crops in the tropics. The fungi are listed alphabetically by perfect state genera with, occasionally, the omission of entries under the imperfect state genera better known to many plant pathologists. A description of the morphology and more general features of each genus is followed by a list of main references to the genus. For each species, under each genus there is a description of the morphology and a critical reference followed by accounts of the diseases it causes — again accompanied by a list of important references, each including the volume and page or abstract number for an abstract in the *Review of Applied Mycology* or the *Review of Plant Pathology*; this is particularly useful. The accounts are full and remarkably compact for the amount of information they contain. Quite properly, the emphasis is on factors relevant to diseases in the field and on their control. But there are quite adequate references to physiological, biochemical and related research.

There is an excellent appendix of host plants, their major and minor pathogens, and selected references, a list of common and botanical names of host plants and, finally, two indexes of fungi under perfect and conidial state names.

This book presents a huge amount of information accurately, clearly and systematically, is an excellent work of reference and will remain so for many years. The accounts of the diseases are such that they can also be read with pleasure. Expensive though it be, a thousand copies could be bought for the price of one electron microscope and, where most needed, they will be a lot more useful. They will also require little maintenance and last much longer. □

R. K. S. Wood is Professor of Plant Pathology and Head of the Department of Pure and Applied Biology at Imperial College, University of London.

Topical tropical meteorology

Reginald Newell

Climate and Weather in the Tropics. By H. Riehl. Pp.611. (Academic: 1979.) £28, \$67.50.

STRANGERS to our subject may wonder why the tropics should be accorded a separate treatment in studies of climate and weather. After reading Riehl's book, I suspect that it is the sheer volume of new material available rather than fundamental differences in the characteristic phenomena that prompts this division. Many of the early meteorological studies dealt with the tropics; Trade Winds were analysed for their practical use to shipping, and early ideas about the global general circulation were based (often erroneously) on the tropical Hadley cell circulation.

In the twentieth century, frontal systems and the changing patterns of cyclones and anticyclones began to be explored in depth, both observationally and theoretically, and the focus and excitement switched to middle latitudes. The old ideas of general circulation were replaced by those which gave a pre-eminent role to the large-scale quasi-horizontal eddy processes; theoretical work by Jeffreys was followed by observational studies by Bjerknes, Priestley, and Starr and his colleagues. Most of the observational data came from the middle and high latitudes of the Northern Hemisphere. During and after the Second World War, forecasters from middle latitudes had to ply their trade in the tropics and many tried to translate ideas about middle-latitude systems to those of the tropics, without success. In fact, when data on the general circulation of the tropics became available it was realized that the quasi-steady Hadley cell circulation and the associated Walker circulations in longitudinal planes dominate the tropics, while the eddy processes govern the general circulation of middle latitudes.

There are of course some major differences between the regions: the rather flat surfaces of constant potential temperature in the tropics compared to the steep slopes — the strong baroclinicity — of middle latitudes; the easterly jet streams rather than the westerly jets of middle latitudes; the presence of a two-year cycle, with a shift from east to west, in the zonal winds of the tropical lower stratosphere as compared with the one-year seasonal cycle of middle and high latitudes; the large extent of tropical warm ocean, with temperatures greater than about 300 K, which seems to be necessary to spawn and maintain hurricanes; the mean vertical motions which in monsoon regions reverse from winter to summer rather than every few days as in middle latitudes; the dominance of the Earth's vorticity over the local vorticity in middle latitudes; and the strong vertical coupling which characterizes systems of middle latitudes

and contrasts with the near independence of the lower and upper troposphere in the tropics. In this book, Riehl describes all the important phenomena and places them in the context of the global general circulation. He seems to have read all that has been written on tropical meteorology and provides an excellent set of references. A notable feature is the inclusion of findings from recent expeditions to the tropics such as the Tropical Atlantic Experiment.

The problems of how energy is transported vertically in the tropics from its source, effectively at the sea surface, and how energy gets into the atmosphere from the warm ocean — both topics that Riehl has contributed to significantly — are dealt with extensively and there is ample material for students to carry the subjects further.

Tropical cyclones and the various ideas about their structure, mechanisms, formation and movement are covered in detail and occupy about one quarter of the book. There is a well-written chapter by Ferdinand Bauer on numerical prediction of hurricanes.

My only major complaint — from which Bauer's chapter is exempt — is that numerous typographical errors are scattered throughout the book; together with the awkward construction of some phrases and poor standardization of units, they often make the text hard to follow. A firm editorial hand needs to be applied before the next edition. Nevertheless, Riehl's book provides a unique source of information and deserves to be read by all meteorologists and to be the standard reference for many years. □

Reginald Newell is a Professor of Meteorology in the Department of Meteorology and Physical Oceanography at the Massachusetts Institute of Technology.

tRNA 2

J.P. Goddard

Transfer RNA: Biological Aspects. Edited by D. Söll, J.N. Abelson and P.R. Schimmel. Pp.578. (Cold Spring Harbor Laboratory: 1980.) \$70, \$84 outside USA.

THE publication of this book follows approximately six months after that of its companion volume *Transfer RNA: Structure, Properties and Recognition* (reviewed in *Nature* 284, 287; 1980). The format of the two volumes is similar. Thus the contributors, who were participants at the Transfer RNA Meeting at Cold Spring Harbor in August 1978, have given us here a series of readable, authoritative and objective articles covering all the biological aspects of tRNA, except synthetase recognition, aminoacylation and ribosome

interaction which were dealt with in the first volume. Division of the contributions into four sections — biosynthesis, gene arrangement, suppression and coding, and "other roles of tRNA" — is perhaps the best in the circumstances. However, the organization and expression of tRNA genes are so intimately linked that articles in the first two sections overlap and some of them also impinge upon suppression which has proved such a useful property in study of tRNA transcription and processing.

The opening section on tRNA biosynthesis begins, as do most sections in both volumes, with a useful general review of the topic by the session chairman at the meeting. There then follow a dozen shorter articles in which specific genetic and enzymological studies are thoroughly covered. Gene arrangement is introduced by a beautifully succinct review. The topics of the articles that follow, though broad-ranging, are somewhat unbalanced with four articles on *Drosophila*, including three on *in situ* hybridization, but none on tRNA genes in mitochondria or in *Xenopus*. No general reviews introduce the final two sections of the book. While a brief overview of the rather esoteric topic

of suppression would have been most useful, the diverse topics of the final section could only be dealt with satisfactorily by individual articles. The volume concludes with three appendices on tRNA gene localization and codon usage.

One final comment should be made on the two-year delay in publication which, in some respects, is fortunate rather than regrettable. In mid-1978 there were tantalizing promises of rapid advance in some of the newer fields of study, such as tRNA gene organization in eukaryotes and removal of intervening sequences from tRNA precursors. The contributors have made an admirable job of updating their articles — of the 1300 references cited, nearly 200 date from 1978, 75 from 1979 and 16 from 1980. Thus most of the discoveries in the year following the 1978 meeting are reported in this volume.

The book has all the qualities one has come to expect from Cold Spring Harbor monographs and should find an appreciative readership among workers in tRNA research and in molecular biology generally. □

J.P. Goddard is a Lecturer in Biochemistry at the University of Glasgow.

scientific or rigorously thematic, which is reflected by some unevenness, with certain elements sitting less easily under the general title. There are also clear differences in scientific content, philosophy and merit among the various contributions, including some which are trivial or frankly eccentric. However, on balance this is a fitting tribute to Jean Maetz, which should prove valuable to fish physiologists and epithelial transport workers. □

Clive Ellory is a Lecturer in Physiology at the University of Cambridge.

Epithelial physiology: tribute to Jean Maetz

Clive Ellory

Epithelial Transport in the Lower Vertebrates. Edited by B. Lahlou. Pp.366. (Cambridge University Press: 1980.) £25, \$55.

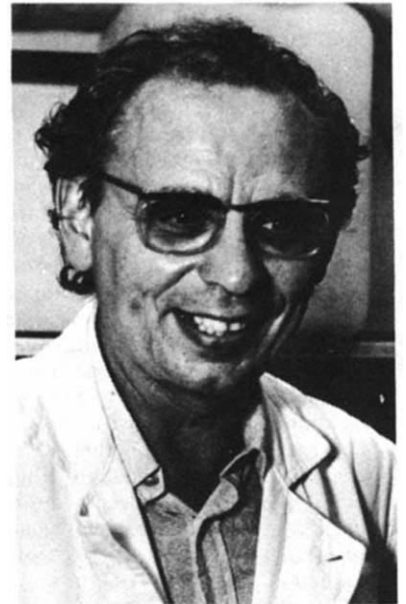
THIS book represents a memorial symposium held as a tribute to Jean Maetz by many of his friends, colleagues and collaborators. It was a meeting Jean would certainly have enjoyed, the emphasis being on fish and amphibian epithelial physiology, with many authors concentrating on endocrinological aspects. Aside from the obvious environmental attractions of working in Villefranche, Naples or Woods Hole, physiologists have become increasingly interested in fishes for studying the mechanisms of epithelial secretion. Fish gills and opercular skin, flounder intestine and shark rectal gland are all preparations which have generated valuable models and influenced thinking on epithelial function. These systems exercised Jean Maetz's considerable talents and represent the principal content of the present volume.

Papers on fish gills comprise half the total contributions. Thus, gill structure and function are covered well in the initial section, with emphasis on vasculature and functional morphology. The importance of the chloride cell in gill physiology is apparent from the number of times this topic recurs in the book, and from the descriptions of the exciting, isolated skin preparations from *Fundulus* and

Gillichthys. New results, including data on gill permeability to large molecules, and some detailed electron microscopy produce tentative functional models for the chloride cell. Other gill transport systems beside Cl covered in the book include Na^+/NH_4 exchange and calcium transport. There are good biochemical presentations on gill Na, K-ATPase and the more controversial anion-stimulated ATPase. A salutary lesson is presented in the Na,K-ATPase chapter, where vanadate, a specific inhibitor of this enzyme *in vitro*, was found to be functioning on gills *in vivo* as a vasoconstrictor.

Kidney is mostly approached from an endocrinological point of view, although there are two impressive studies on single nephron physiology which emphasize the widely different roles for the kidney in marine and freshwater fish. Intestinal functions discussed include salt and amino acid transport, and amphibian skin is represented by three contributions mostly concerned with epithelial sodium transport. Endocrinological factors are emphasized in many of the contributions, but there are two small reviews dealing specifically with the hormonal control of ion transport.

Overall, there is much of interest in this book. Selection criteria for participants in such a memorial symposium obviously include factors which are not strictly



Jean Maetz, a talented physiologist

Birds in brief

Robert Hudson

A Complete Checklist of the Birds of the World. By Richard Howard and Alick Moore. Pp.701. (Oxford University Press: 1980.) £17.50, \$49.50.

THIS book comprises a straightforward listing of the living birds of the world, giving for each species the scientific and selected English name and a brief indication of geographical range; for polytypic species, named subspecies are also listed. Essentially, it is a condensed and partly updated version of the authoritative 15-volume *Checklist of the Birds of the World* edited by J.L. Peters and others (Harvard University Press, 1931–1979), but lacking the taxonomic and nomenclatorial comments, references and detailed distribution notes to be found in the larger work. The introduction to the present book suggests its major use will be as a handy personal checklist for the

widely-travelled birdwatcher. Perhaps, but I will find it more useful as a quick reference for identifying unfamiliar bird names encountered in the serial literature, and in this respect its 59-page index is admirable.

Specialists in particular bird groups will not find it hard to quibble with points of detail, but on the whole the compilers offer a reasonable compromise between the often conflicting treatments to be found in

avifaunal publications. Several other books of this type have appeared since 1974, the present one being the most comprehensive and therefore the most costly. It is the only one to include subspecies; but this is the weakest aspect of the book, since its authors are not taxonomists. A simplified listing of racial names gives a misleading impression of subspecific rigidity (whereas clinal situations are common even within land areas as small as

the British Isles); moreover, named races present a continuum from well-differentiated semi-species to those hardly worthy of recognition, though this basic truth is not made evident to the reader. Thus, this is a potentially useful book, but not a definitive work.

Robert Hudson is on the scientific staff of the British Trust for Ornithology, and is editor of the Trust's quarterly journal Bird Study.

Semiconductor lasers come of age

S. D. Smith

Physics of Semiconductor Laser Devices. By G. H. B. Thompson. Pp.549. (Wiley: 1980.) £22.75, \$68.25.

THE semiconductor diode laser based upon the radiative recombination of carriers injected across a p-n junction is now nearly 20 years old from the date of the original concept. It has developed from a laboratory speciality operating only at cryogenic temperatures into a range of optoelectronic devices of application in optical fibre communication, military systems, printing, infrared spectroscopy, pollution detection and short-pulse work, to mention only part of the list.

This excellent and comprehensive book opens with a most readable chapter outlining the history, concepts and basic principles, without the use of mathematics, emphasizing the dramatic effect of the introduction of hetero-structures, using layers of alloy material, which separately confine the injected carriers and guide the generated optical waves with favourable effects on threshold current and temperature of operation. In the next 100 pages the author discusses the basic processes of light emission and laser action, and includes appropriate development of absorption, stimulated and spontaneous processes, band structure in the presence of heavy doping, and recombination times. Gain-current relationships, threshold currents and efficiencies are calculated and compared with experimental results for the basic or homostructure p-n junction laser with a simple resonator, for which the mode properties are also given. The treatment owes something to the earlier *Semiconductor Lasers and Light Emitting Diodes* by Kressel and Butler (Academic, 1977), but is more continuous and complete at the expense of rather intricate notation. I found this section a useful text for part of a graduate course on optoelectronic devices held at the Optical Sciences Center of the University of Arizona.

Later chapters deal with heterostructure lasers, optical waveguides and all the many variations of laser geometries in considerable detail. Methods of fabrication and the physics of dynamical response are also considered.

The only notable omission is the absence of discussion in any detail of the lead salt family of diode lasers and their application in high resolution infrared spectroscopy, perhaps not surprising since the book concentrates on the GaAs alloys. However, the basic physical principles for all types of semiconductor diode lasers are well covered which is a stated aim of the book. The author does not claim to be comprehensive in citing the literature — a difficult task in this active field — but nevertheless provides copious references in each chapter.

The book frequently examines the relation between semiconductor lasers and

other types of lasers — emphasizing the high gain values, for example. It is thus easy to see what semiconductor injection lasers have not achieved — visible room temperature operation, high powers or wide-band tuning in one device.

With the slight qualification that the book's title is not quite consistent with its concentration on the GaAs injection diode laser family, it is to be generally recommended for a variety of uses in research, application and teaching.

S. D. Smith is Visiting Professor at the Optical Sciences Center, University of Arizona, Tucson, and Head of the Physics Department at Heriot-Watt University, Edinburgh.

Organic infrared-structure correlations

P.C. Crofts

Infrared Characteristic Group Frequencies. By G. Socrates. Pp.153. (Wiley: 1980.) £24, \$72.

INFRARED spectroscopy is perhaps the most generally applicable and widely used physical method for investigating structures of organic compounds. Although qualitatively rationalized by classical physical theory, it is an essentially empirical activity in which absorption bands reveal individual chemical bonds or larger submolecular units whose vibrations interact with radiation of the observed frequencies. Users of the technique thus require comprehensive collections of assignments of recorded absorption frequencies to particular functional groups or other structures. Although described in the preface as "a simple introduction to characteristic group frequencies", this book is a substantial and valuable work, simple only in its arrangement and empiricism and not in any restriction of its subject matter.

An introductory chapter contains three successively more detailed charts of the range 4000–200 cm⁻¹; the first shows regions without absorptions and infers absences of particular groups, the second relates observed bands to possible

vibrations, and the third shows absorption regions for many specified compound types. This is followed by 19 chapters dealing with individual substituents of particular importance (e.g. O–H, C=O), with collections of related substituents (e.g. triple-bonded groups, halogen compounds), or with larger structural features (e.g. alkane residues, six-membered heterocycles). A short section surveys X–H stretching overtones in the 1400–4000 cm⁻¹ region. Each chapter contains extensive tables of absorption bands, in wavenumber and wavelength units, with well-written explanatory text and many references.

A compilation of this type can be only a starting point in an infrared investigation — which also requires comparison with reference spectra of model compounds — but when used intelligently this book should point many investigators towards solution of their problems. It is well printed, with wide, short, double-column pages which are convenient when examining a spectrum, and is recommended to all who use infrared spectroscopy in structural investigations.

P.C. Crofts is a Senior Lecturer in Chemistry at the University of Manchester Institute of Science and Technology.

CORRESPONDENCE

Continued from page 742

anomalies in the data very successfully. For instance, he showed that in the Cornish sample the biggest single annual decline in the incidence of herd breakdowns was from 5.5 to 3.2 per cent in 1975-76 (a 42 per cent decline). Yet by 31 August 1976 only ten "fire-brigade" cases had been completed in Cornwall¹, an area of approximately 355,000 hectares (a fire-brigade action normally involves the removal of a small number of badger social groups around a farm with a herd breakdown). Is Dr Yates telling us that this 42 per cent decline in Cornwall was the instant result of ten completed fire-brigade actions? If ten fire-brigade actions could be so successful, it is surprising that the TB problem was not solved long ago. In other areas a similar situation can be seen, with significant reductions in the number of herd breakdowns before the onset of badger gassing, or before effective measures could have been achieved by the badger gassing programme. The data are not conclusive, but there is good evidence to suggest that the incidence of TB was undergoing a decline before the onset of gassing, and that to attribute the decline solely to the gassing campaign is not justified.

Dr Yates also contested that only 15 per cent of the Cornish herd breakdowns could be attributed to badgers, and "presumed" that badgers were responsible in many more cases. Lord Zuckerman (*Nature* 19 February 1981, p.628) also contested this point, saying that badgers in Cornwall have only been routinely investigated as a possible source of infection since 1974. However, his figures still demonstrate our original point; even since 1974 badgers were still only believed responsible for a minority of breakdowns, and even these figures are very dubious. In his letter Lord Zuckerman states that the majority of herd breakdowns in Cornwall supposed to have originated from badgers were attributed to infected badgers living up to two miles from the breakdown, and 28 per cent were attributed to infected badgers found more than two miles away. But in areas of relatively high badger density, such as Cornwall, badger territories are small, about 75 hectares (report, p.37). Ministry of Agriculture, Fisheries and Food (MAFF) studies in Gloucestershire² showed that in an area of high badger density, itinerant badgers were rare, and long distance movements outside the group territory were not recorded. The available information suggests that in Cornwall badgers are very unlikely to make contact with a cattle herd two miles away. Lord Zuckerman might as well tell us that in 100 per cent of cases an infected badger was found within twenty miles; such a figure is no less meaningful than those presented by Lord Zuckerman.

Figures such as those discussed are of little value if we do not understand how and where transmission takes place. As we said in our original letter, the mere presence of infected badgers does not always result in herd breakdowns. In his letter, Lord Zuckerman states that transmission of TB from badgers to cattle occurs in pasture that has been contaminated with sputum, pus, urine and faeces. It has never been proven that this is the sole point of transmission, nor even the major

point of transmission, and this is another area in which further research is needed. As long ago as 1974, MAFF staff³ pointed out that "On many occasions badgers have actually been observed in cattle buildings, presumably in search of food, and contamination of cattle food concentrates may also be important [in transmission]" and "On a number of occasions farmers have reported finding badgers in farm buildings eating cattle feeding stuffs". In his report, Lord Zuckerman (p.35) said that badly diseased badgers may leave their sett and take up refuge in farm buildings. How common is such behaviour? Further investigation might show that farm buildings are a major point of transmission, and if so a significant reduction in herd breakdowns might be achieved by a simple improvement in animal husbandry. Actions such as these to complement the gassing programme do not seem to have been considered by Lord Zuckerman; we feel that they should be.

Lord Zuckerman asserts that during the period of the moratorium (October 1979 to October 1980) the increase in the percentage of reactor herds in the affected counties of the South West was a direct result of halting badger control measures. This assumption was supported by Dr Yates in his letter, where he showed that the incidence of TB in badgers increased immediately following the moratorium on gassing. This not only totally ignores the likelihood of natural cyclic trends in TB prevalence, as discussed in our original letter and the reply by Dr Yates, but also requires us to believe that following the moratorium there was an immediate increase in the badger population, and consequently of the numbers of badgers infected with TB. It must be remembered that although no new areas were gassed during the moratorium, gassing was continued "to maintain freedom from the disease in areas already cleared of it"². Since the moratorium only applied to areas not previously gassed, it seems reasonable to presume that, in the absence of any evidence to the contrary, the badger population was fairly stable in those areas. Badgers are long-lived animals with a relatively slow reproductive rate. Since the moratorium only lasted one breeding season, even if there was a population increase it could only have been small. And even presuming that there was an upward trend in badger population density during the period of the moratorium, there is no unequivocal evidence that TB in badgers is density dependent. Lord Zuckerman's report (p.68, 70) shows that TB is not only prevalent in badgers in areas of high density, but also in areas such as South Dartmoor, where the badger population is very low. Also, when considering Lord Zuckerman's and Dr Yates' arguments, it is necessary to take account of the rate of development of the disease in badgers. Experiments by MAFF scientists suggest that this may take several months¹. In affected areas of the South West, tuberculin testing of cattle is carried out annually³, and so there will be a further delay between possible infection of cattle and their subsequent discovery at tuberculin testing. Clearly there is no evidence to support Lord Zuckerman's and Dr Yates' argument that TB in badgers and cattle immediately increased as a result of the

moratorium on gassing, and it is not surprising that an increase in TB in badgers was not seen in all areas, the point we made in our original letter.

Much of Lord Zuckerman's latest letter deals not with the TB problem, but is an attack on the Mammal Society and some of its members. Lord Zuckerman questions the right of the society to discuss the subject, since he suggests that the society is not fully competent in all the relevant fields of expertise. While this is a debatable point, no one has ever questioned Lord Zuckerman's right to express his opinion.

Also, Lord Zuckerman is unfamiliar with the aims or constitution of the Mammal Society, and so it is necessary to correct some more misleading statements. We are not a conservation society, and such interests are outside our remit. However, at our AGM on 14 April 1973, a motion from the membership mandated the Council:- (1) "to encourage the discussion of contentious issues [relating to the study of mammals] with the aim of showing where more information is needed in order to reduce the uncertainties of the issue", and (2) "to write, when appropriate, general letters asking for action or expressing concern". These are quotations from the minutes of that meeting, and clearly we have acted in accordance with the wishes of our membership. Lord Zuckerman's lengthy discussion on whether other societies can act in a similar manner is totally irrelevant. And of course our statements are not anonymous; they have been made by the officers of the Mammal Society, and a full list of elected officers is published.

The Mammal Society hopes that the badger/TB problem can be solved soon, and that some means can be found whereby the incidence of TB in cattle in the South West can be permanently reduced. We still feel that this aim can only be achieved by a critical analysis of all the data. We feel that the Zuckerman report does not help solve this complex problem; the only statistical treatment presented in the report is the calculation of percentage changes. This is clearly unacceptable for a complex epidemiological problem. Since the data are not conclusive, and since none of our original points have been answered satisfactorily, we suggest that the Minister of Agriculture should establish a Scientific Advisory Group, containing a small number of independent scientists such as wildlife epidemiologists and statisticians, who can analyse the data in detail and advise the Consultative Panel on Badgers and Tuberculosis, which currently lacks such expertise.

J. R. FLOWERDEW
(Hon. Sec.,
Mammal Society)

Reading, Berks, UK

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